Alkaptonuria and Ochronosis

Jozef Rovenský
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Introduction

1

Jozef Rovenský

Workers of the National Institute of Rheumatic Diseases (NIRD) in Piešťany prepared the first monograph in world literature called *Alkaptonuria and ochronosis* that was published by SAV publishing house in Bratislava in 1956. The authors of this original book were as follows: Professor Štefan Siťaj, MD, DSc., Professor Ján Červeňanský, MD, DSc., and Associate Professor Tibor Urbánek, PhD. In their practice, the next generations of physicians and scientific workers of NIRD Piešťany paid a lot of attention to serious diseases like alkaptonuria and ochronosis because their incidence in Slovakia is extraordinarily high. On the occasion of the 20th anniversary of the death (September 1990) of the founder of Slovak rheumatology, Professor Štefan Siťaj, MD, DSc., the authors decided to prepare a new supplemented edition of this monograph. The book was enriched with the knowledge gained during those 20 years with particular respect to diseases of metabolism of aromatic amino acids, genetics and the use of more recent imaging methods for the diagnostics of alkaptonuria and ochronosis. It was our intention to publish a monograph that would contribute to clinical enrichment of the physicians and scientific workers in the field of alkaptonuria and ochronosis.

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Alkaptonuria and Ochronosis

2

Zbyněk Hrnčíř

The very interesting topic of alkaptonuria including metabolism of proteins and particularly of aromatic amino acids has been reviewed in this book on the basis of largely the authors' own novel observations. The fact that the whole work is based on entirely original, own material conveys so much new information and simultaneously points out so many issues that the book deserves to be published in every world language because such a publication would become the source of many papers for authors worldwide. There is nothing to criticise in any part of the book even if the most stringent review is applied, and I can only heartily recommend it. I believe no other publication in Slovak scientific literature has the same standard from the point of view of international and world significance as the work of Siťaj, Červeňanský and Urbánek. Therefore, it deserves the best standards of publication possible.

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History

3

Jozef Rovenský and Mária Stančíková

Content

AKU is an ancient disease; scientists have found evidence of alkaptonuria in the Egyptian mummy Harwa dated 1500 B.C. However, the term alkaptonuria (AKU) was used for the first time in 1859 in a female patient who had a reducing compound detected in urine. Later on, this compound was identified as homogentisic acid. In 1890, the English physician Archibald Garrod examined the urine of a 3-month-old boy – it was yellow brownish and Dr. Garrod diagnosed alkaptonuria. At that time, it was assumed that alkaptonuria was induced by a bowel bacterial infection. However, Garrod also examined the parents of the boy, and he found out that they had a close family relationship (cousins). After examining several patients and their families, he concluded that the answer to his questions was provided in the work of Mendel on inheritance that had appeared in England at that time. In 1902, Garrod proposed the hypothesis that alkaptonuria is an inherited metabolic disease, and the lack of the enzyme which degrades homogentisic acid is the result of a defective gene. Later on, this conception proved to be true. His article on alkaptonuria was published in the Lancet (Garrod 1908). At that time, it was a daring statement when we realise how little was known of enzymes, human genetics

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and intermediary metabolism. His knowledge was summarised in the book called 'Inborn Errors of Metabolism' (Garrod 1909). For his scientific work, he became a member of the Royal Society, and he was knighted in 1918. Almost half a century later in 1958, La Du published biochemical proof of the defect in AKU (La Du et al. 1958). He demonstrated the absence of activity of 1,2-dioxygenase of homogentisic acid in liver homogenates from a patient with AKU, and he found out that the defect was associated with one enzyme. He assumed that affected persons do not synthesise this enzyme. The gene responsible for AKU was localised in 1993 by Pollak et al. in chromosome 3 (3q2) (Pollak et al. 1993). Siťaj, Červeňanský, Urbánek, Hüttl and other co-workers significantly contributed to the knowledge of clinical manifestations of alkaptonuria and ochronosis (Siťaj 1947, 1977; Urbánek and Siťaj 1955; Siťaj et al. 1956; Hüttl et al. 1966). In 1947, Siťaj diagnosed and described the first case of alkaptonuria (Siťaj 1947), and by 1953, he and his co-workers had collected a set of 102 patients, while there were only about 100 predominantly sporadic cases described worldwide at that time. Slovakia and the Dominican Republic were presented in the world literature as the countries with the highest incidence of alkaptonuria (1:19,000 people). One case per 250,000 to 1 million people is given worldwide (Phornphutkul et al. 2002). Siťaj, Červeňanský and Urbánek dealt with the description of ochronosis and its development in patients with AKU as well as with its detailed clinical manifestations in joints. The results were published in the monograph *Alkaptonuria and ochronosis* (1956) – the first one in the international literature. Their pioneering works are still quoted. In 1968, Sršeň and his co-workers (Sršeň and Neuwirth 1974; Sršeň 1984; Sršeň and Sršňová 1996; Sršeň et al. 1996, 2002) from the Research Laboratory of Clinical Genetics in Martin continued their epidemiological and genetic studies in patients suffering from AKU in Slovakia. The following institutions participated in the molecular characterisation of mutations in the Slovak population: the Institute of Molecular Physiology and Genetics of Slovak Academy of Sciences in Bratislava and Faculty of Natural Sciences of Comenius University in Bratislava (Zaťková et al. 2000a, b, c). The majority of the followed families came from the locations in Slovakia that were studied by Siťaj et al. and later on by Rovenský with his co-workers. Genetic aspects were studied by Bošák in cooperation with institutions in Bratislava (Rovenský and Urbánek 2000, 2003; Rovenský et al. 2000; Zaťková et al. 2000a, b, c). Current diagnostic innovations contributed to the characterisation of alkaptonuria as a separate diagnostic entity. Magnetic resonance imaging can display a thickening of the Achilles tendon; CT scans and echocardiography can reveal coronary arterial calcification or injury of the heart valve. Prostate stones in ochronosis can be visualised by X-ray, and kidney stones can be detected by ultrasonography. Biochemical analysis of urine shows increased excretion of telopeptide fragments of collagen (NTx) suggesting increased bone resorption. The results of molecular genetics enable DNA diagnostics of AKU which is considered the definitive diagnosis of the disease and represents qualitatively the highest degree of diagnostics.

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Metabolism of Aromatic Amino Acids

4

Mária Stančíková and Jozef Rovenský

Content

Phenylalanine and tyrosine are the simplest aromatic amino acids derived from alanine. Phenylalanine is an essential amino acid that our body cannot synthesise. This does not apply to tyrosine, which the body can synthesise only if there is a sufficient amount of phenylalanine – and availability of the enzyme participating in conversion of phenylalanine to tyrosine (Fig. 4.1). There is a close mutual relationship between phenylalanine and tyrosine; phenylalanine converts to tyrosine in the liver and to phenylpyruvic acid in the kidneys. Aromatic amino acids have a common intermediary metabolism, and conversion of phenylalanine to tyrosine and its further metabolism is controlled by a complicated enzymatic system. Disorder of the conversion of phenylalanine to tyrosine is called phenylketonuria, and it is one of the most frequently occurring recessive inherited diseases (approximately 1 patient per 10,000 newborns). In phenylketonuria, either the phenylalanine hydroxylase that converts phenylalanine to tyrosine (Fig. 4.1) or its cofactor tetrahydrobiopterin is absent (Blau et al. 2005; Crone et al. 2005). Treatment of children with phenylketonuria has to start in the 3rd month of life, because unrecognised phenylketonuria results in mental retardation, seizures, excessive tremor and hyperactivity.

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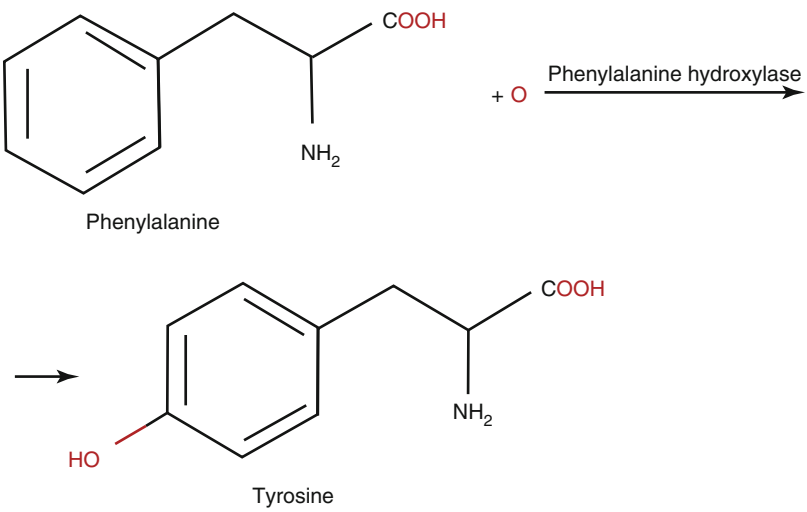

Fig. 4.1 Conversion of phenylalanine to tyrosine

Phenylalanine has to be considerably reduced in food, and on the other hand tyrosine has to be added. Several types of hyperphenylalaninaemia that do not fulfill the criteria of the classical forms of phenylketonuria have been revealed during the examinations aimed at early diagnostics of phenylketonuria. In the case of the mild form of phenylketonuria, the body tolerates a higher intake of phenylalanine in food when compared to typical phenylketonuria. Tyrosine plasma concentration also increases. In cases of transient phenylketonuria, clinical and biochemical signs regress or completely vanish. In a special form of hyperphenylalaninaemia, without ketonuria, phenylalanine plasma concentration is slightly increased; however, phenylpyruvate is not excreted. After phenylalanine intake, its concentration increases; then it decreases, and tyrosine concentration can also increase. In both forms, decreased activity of phenylalanine hydroxylase to 10–20 % of normal values has been demonstrated. Disorders of phenylalanine metabolism are also present in Hartnup disease and generalised aminoaciduria. Approximately 90 % of phenylalanine in the body converts to tyrosine. The remaining 10 % of phenylalanine is used for protein synthesis. Tyrosine, which forms from phenylalanine under normal circumstances, can further be metabolised via various pathways with the formation of:

1. Fumarate and acetoacetate
2. Dopamine, adrenaline and noradrenaline
3. Melanins
4. Thyroid hormones – thyroxin and triiodothyronine

Degradation of tyrosine to fumarate and acetoacetate starts with transamination of tyrosine (Figs. 4.2 and 4.3) to para-hydroxyphenylpyruvic acid, while a-ketoglutarate is the cofactor. The change of the lateral chain to the ortho position, oxidative

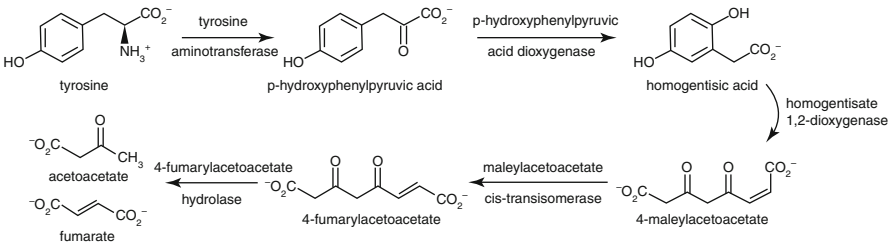

Fig. 4.2 Degradation of tyrosine to acetoacetate and fumarate

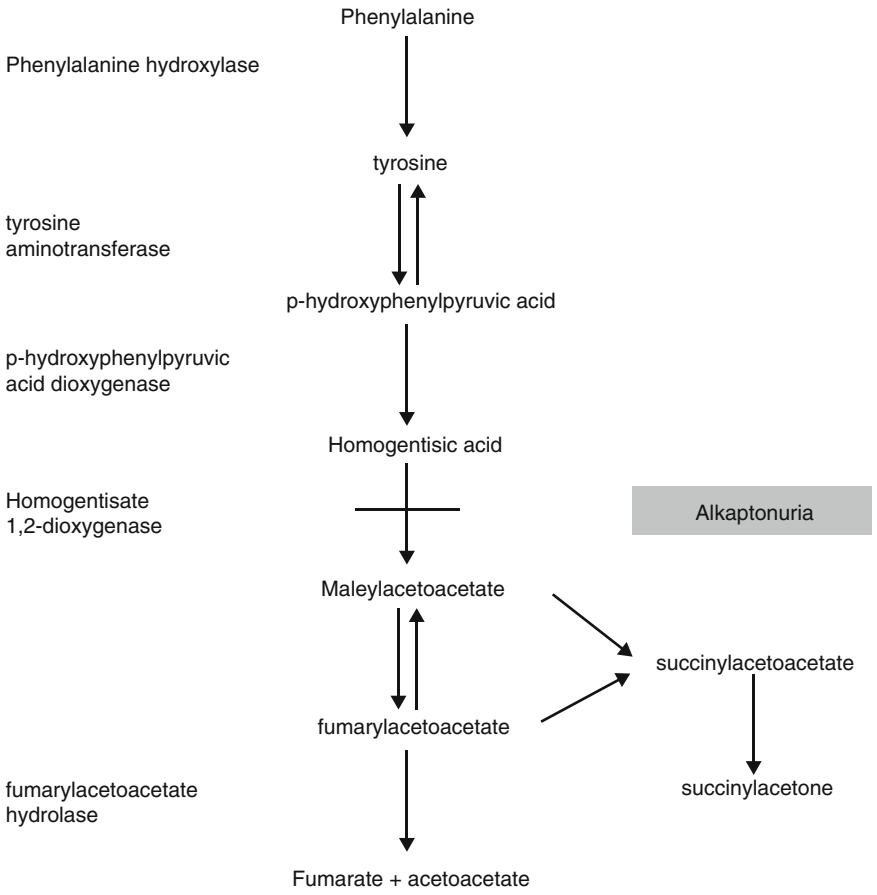

Fig. 4.3 Metabolic pathway of phenylalanine and tyrosine degradation. Disorder of conversion of homogentisic acid to maleylacetoacetate, cause of alkaptonuria

decarboxylation and hydroxylation results in the formation of homogentisic acid. The next step of tyrosine metabolism is degradation of homogentisic acid.

The aromatic ring of homogentisic acid is opened up by means of homogentisate 1,2-dioxygenase with the participation of an oxygen molecule with subsequent formation of maleylacetoacetate. Fumarylacetoacetate forms by means of maleylacetoacetate cis-trans-isomerase and rotation of the carboxyl group arising from the hydroxyl group. Glutathione serves as cofactor in this step. Fumarylacetoacetate is subsequently degraded by fumarylacetoacetate hydrolase with the participation of a water molecule to the final products – fumarate and acetoacetate. These products enter the metabolic pathway of citric acid. Another pathway of tyrosine conversion is formation of dopamine and catecholamines. By means of tyrosine hydroxylase, tyrosine converts to DOPA-3,4-dihydroxyphenylalanine. Dopamine forms by means of DOPA-decarboxylase. Subsequently adrenaline and catecholamines that have the role of neurotransmitters form from dopamine. Tyrosine can also be metabolised to melanins that protect us against UV radiation. Tyrosine oxidises in the presence of oxygen to DOPA. DOPA dehydrogenates with the formation of dopaquinone. Further reactions result in the formation of indolequinone (indole-5,6-quinone) via several intermediate products. Brown-black eumelanin arises by its polymerisation. Eumelanin is the pigment found in hair, eyes and skin. Dopaquinone can also bind SH-groups of cysteine and further be oxidised to polymeric quinones. Yellow pheomelanin forms by this pathway. Tyrosine is also part of thyroglobulin (the protein found in the thyroid gland), thyroxine and triiodothyronine. These thyroid hormones have multiple effects; in general, they accelerate reactions taking place in cells.

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Tyrosine Metabolism Disorders

5

Jozef Rovenský and Mária Stančíková

Contents

The most frequent tyrosine metabolism disorders (Rezmani 2007; Dionisi-Vici et al. 2000; Held 2006) are as follows:

- Tyrosinaemia (type I, II, III)
- Transient tyrosinaemia of the newborn
- Hawkinsinuria
- Alkaptonuria

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5.1 Tyrosinaemia Type I (Tyrosinosis)

The defect is caused by absence or considerably decreased activity of fumarylacetoacetate hydrolase resulting in elevated levels of plasma tyrosine and accumulation of tyrosine metabolites, especially succinylacetone. The disease can have an acute or chronic course. In cases of acute tyrosinosis of infants, they die within 6–8 months due to hepatic failure, if they are not treated. In the chronic form, cirrhosis develops as well as renal injury and involvement of peripheral nerves. Hepatomas are frequent. Tyrosinaemia type I is a genetic disease with autosomal recessive inheritance; therefore, hepatic and renal injury cannot be removed completely. However, the signs can be mitigated by food with low content of tyrosine and phenylalanine. Nitisinone (Orfadin) has been recently authorised for the treatment of tyrosinaemia type I. This treatment together with restrictive diet can successfully reduce disease symptoms, and it enables the children to develop and live normally.

5.2 Tyrosinaemia Type II (Richner-Hanhart Syndrome)

The defect is caused by deficiency of cytosolic hepatic tyrosine aminotransferase, whereas the mitochondrial isoenzyme has normal activity. Enzyme defect results in elevated plasma levels of tyrosine and its elevated urine concentration. Tyrosine is the only amino acid that is elevated in urine. Clinical manifestation is characterised with multiple malformations (microcephaly, corneal changes), erythema, palmar and plantar hyperkeratosis and disorder of mental development. In contrast to tyrosinaemia type I, hepatic and renal functions are normal. The therapy consists of lower intake of aromatic amino acids and proteins. This diet results in normalisation of the concentration of tyrosine and tyrosine metabolites in plasma and urine. Early initiation of the treatment prevents the changes on eyes and skin. The disease has autosomal recessive inheritance.

5.3 Tyrosinaemia Type III

Tyrosinaemia type III has autosomal recessive inheritance. Dysfunction of p-hydroxyphenylpyruvate dioxygenase in the liver and kidneys leads to neurologic disorders and mental retardation of affected persons. This disease is very rare, and it requires restriction of tyrosine and phenylalanine in food.

5.4 Transient Hypertyrosinaemia of the Newborn

The biochemical cause of this anomaly is delayed maturation of liver parenchyma which is associated with delayed maturation of 4-hydroxyphenylpyruvate dioxygenase. The enzyme biosynthesis – and thus the enzyme activity – normally

increases at the end of fetal development; however, it can be delayed in some newborns. Other factors that affect prolonged hypertyrosinaemia in newborns are high content of tyrosine in milk food and lack of vitamin C. Excessive amounts of ingested tyrosine as well as lack of vitamin C result in formation of hydroxyphenylpyruvate that inhibits the enzyme in the liver of the newborns. Hypertyrosinaemia of the newborns is considered harmless, and it usually regresses spontaneously during the first weeks of life as soon as the intake of proteins decreases and vitamin C is administered. The disease is found in 30 % of premature babies and in 10 % of mature newborns.

5.5 Hawkinsinuria

This rare metabolic disorder is caused by heterogeneous mutation of the gene for hydroxyphenylpyruvate dioxygenase that results in enzyme deficiency. Various metabolites of phenylalanine appear in urine: p-hydroxyphenylpyruvate, p-hydroxyphenylacetate and a special amino acid called hawkinsin. The disease is manifested in childhood after increased caloric load by acidosis, ketosis, hepatomegaly and transient tyrosinaemia. The condition regresses after restriction of tyrosine and phenylalanine intake. This disease has autosomal dominant inheritance.

5.6 Alkaptonuria

Alkaptonuria is caused by deficiency of homogentisate 1,2-dioxygenase (HGD) – the enzyme that is the part of metabolic pathway of degradation of aromatic amino acids phenylalanine and tyrosine (Fig. 4.3). Homogentisate that is excreted in urine is subsequently oxidised by air oxygen to brown-black pigment called alkapton. Another sign in older age is ochronosis – pigmentation of connective tissues (cartilages). The mechanism of ochronosis development consists in oxidation of homogentisate by polyphenoloxidase with the formation of benzoquinone acetate, which polymerises and binds to macromolecules of connective tissues. Inheritance and clinical manifestations of alkaptonuria are described in the next chapters.

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Detection of Homogentisic Acid in Plasma and Urine

6

Mária Stančíková and Jozef Rovenský

Content

Laboratory proof of alkaptonuria in urine is based on the reducing properties of homogentisic acid. Even routine chemical examination of urine can lead to diagnosis of alkaptonuria on the basis of characteristic colouration with Fehling's solution. In patients with alkaptonuria who excrete 2–3 g of homogentisic acid/day, urine with Fehling's solution acquires a characteristic brown-black colouration even in cold condition. In milder forms of alkaptonuria, these colour reactions occur after warming up.

Older quantitative methods of detection of homogentisic acid in urine utilised the capability of homogentisic acid to reduce silver, phosphomolybdic acid or iodine. The drawback of these methods was the fact that they also detected other reducing compounds present in urine. These faults were manifested especially in case of lower concentration of homogentisic acid in urine of patients with alkaptonuria. Extraction of homogentisic acid with ether and subsequent iodometric detection represented certain improvement. Seegmiller et al. (1961) developed spectrophotometric enzymatic detection of homogentisic acid in plasma and urine using purified oxygenase of homogentisic acid. This enabled the authors to detect specifically

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even 1 μg of acid. Maleylacetoacetate acid formed by oxidation is detected by spectrophotometer at 330 nm. Stoner and Blivaiss (1965) developed relatively simple quantitative detection of homogentisic acid in urine. Homogentisic acid in alkaline environment with the presence of air oxygen forms 1,4-benzoquinone-2-acetic acid. This compound is subsequently conjugated with 2,4-dinitrohydrazine. Formed hydrazone in the presence of alcohol solution of sodium hydroxide creates characteristic lavender colour with absorption maximum at 570–580 nm. Later on, capillary electrophoresis (Presto Elgstoen and Jellum 1997) and gas chromatography (Oláh et al. 2003) were used for the detection of homogentisic acid. A very sensitive method for detection of homogentisic acid using liquid chromatography with amperometric detection was developed by Zoutendam et al. (1976). Recently a method for simultaneous quantification of urinary HGA and tyrosine using reverse phase LC-MS/MS has been developed (Hughes et al. 2014).

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Genetics of Alkaptonuria

7

Vladimír Bošák

Content

Alkaptonuria (AKU) is a rare inherited disease caused by defects of the enzyme homogentisic acid dioxygenase, resulting in the accumulation of homogentisic acid in the body and its excretion in the urine. Its polymer, a yellow-black ochronotic pigment, deposits in cartilage and connective tissue, especially in the skin, visceral organs, eyes and ears causing their pigmentation (ochronosis). From the clinical point of view, the most serious pigmentation is in joints, causing ochronotic arthropathy which usually results in severe disability. It is a degenerative process that primarily affects the spine (causing calcification of intervertebral discs) as well as large joints (Rovenský and Urbánek 2003).

In the catalogue of inherited diseases "Mendelian Inheritance in Man" (MIM), the code for AKU is MIM 203 500 (www.ncbi.nlm.nih.gov/omim/). From the historical point of view, it was the first inherited disease in man, which Sir Archibald Garrod identified as following Mendelian inheritance determined by a simple recessive factor (Garrod 1902). He ranked it among those inherited metabolic disorders caused by the defect of a specific enzyme in spite of the fact that he did not know its molecular nature at that time. AKU is a monogenic disease with autosomal recessive inheritance, i.e. it is determined by one gene that is located on some of the homologous chromosomes of man – autosomes (chromosomes 1–22). In a classical case of this type of inheritance, both parents are usually healthy, but they are carriers

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of a pathological allele (healthy carriers – heterozygotes); the risk of children who are recessive homozygotes is 25 % and they will suffer from the disease. Fifty percent of children are healthy, but they are carriers of the pathological allele and 25 % of children are healthy and they are not carriers. In the case of a marriage between a patient with AKU and a healthy carrier, the risk of affected children increases to 50 %. The first mathematical-statistical proof of autosomal recessive inheritance of AKU was provided by Hogben et al. in 1932 (Hogben et al. 1932).

In 1958, La Du with his co-workers (La Du et al. 1958) found out that the biochemical principle of AKU is based on the absence of biological activity of the enzyme homogentisate 1,2-dioxygenase (HGD) in the liver, thus confirming the original assumption of Garrod that it is a metabolic disorder. HGD (MIM 607 474, older term HGO) is the enzyme that participates in the catabolism of phenylalanine and tyrosine (EC 1.13.11.5). Its molecular weight is 50 kDa and it consists of 445 amino acids. It is specific for homogentisic acid and it does not oxidize similar substances like gentisic acid or phenylacetic acid (Schmidt et al. 1997). The gene that codes for the enzyme HGD was cloned in 1996 (Granadino et al. 1997), starting the era of study of AKU molecular genetics. Currently we know that the HGD gene is a single-copy gene (Fernandez-Canon et al. 1996; Granadino et al. 1997). It consists of 14 exons (coding parts of the gene) and 13 introns (non-coding parts of the gene). The complete length of nucleotide sequence is 54,363 bp (base pairs), whilst the coding part of the gene represents only 3 % of its length (1,715 bp). The length of exons ranges from 35 to 360 bp (Genbank sequence NM 000187.2). Repetitive elements represent approximately 26 % of the gene sequence. HGD gene expression is tissue specific; it is found especially in the liver and kidney, with a much lower expression in the small and large intestine, prostate and brain (Fernandez-Canon et al. 1996). Increased activity in the liver and kidney is attributed to the metabolic activity of these organs. In the brain, HGD probably participates in the degradation of amino acids derived from neurotransmitters which often contain aromatic amino acids. In other studied tissues like blood, germinal epithelium, muscles and salivary glands, significant activity of the enzyme was not found (La Du 1989). In 1994, the HGD gene was localized on the long arm of chromosome 3 in man (3q). In the next years, its localization on chromosome 3 was specified more precisely to 3q13.33 using polymerase chain reaction (PCR) and fluorescence in situ hybridization (Fernandez-Canon et al. 1996).

Molecular genetic studies in AKU brought the surprising finding that the HGD enzyme defect is not caused by only one mutation as it was originally anticipated, but by several mutations that are localized in coding as well as in non-coding parts of HGD gene. The first proof that enzymatic loss in AKU is caused by a mutation within the HGD gene was presented by Fernandez-Canon et al. 1996 in a Spanish population. In 2000, in a set of more than 100 patients coming from several countries worldwide, more than 40 various mutations causing AKU were identified (Beltrán-Valero de Bernabé et al. 1999; Porfirio et al. 2000; Zaťková et al. 2000a, b, c). More recently at least 116 mutations and 33 HGD polymorphisms have been encountered (Introne et al. 2009; Grasko et al. 2009; Abdulrazzaq et al. 2009; Oexle et al. 2008; Zaťková et al. 2000a, b, c; Zaťková 2011; Sakthivel et al. 2014).

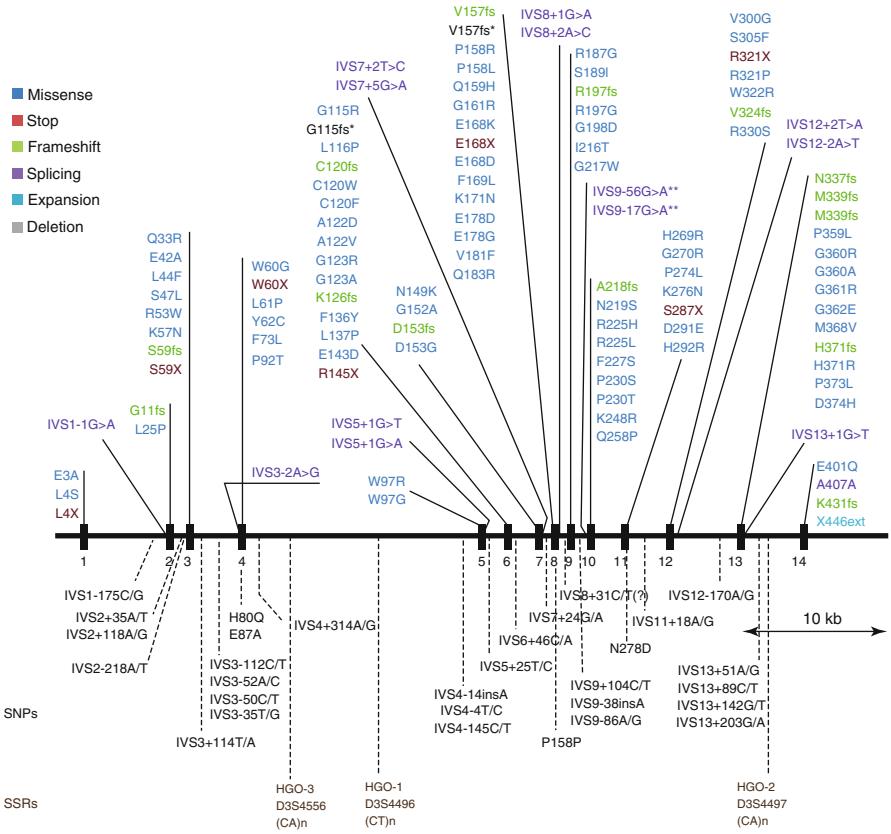

Fig. 7.1 Distribution of AKU mutations in the HGD gene (Zaťková 2011). *1–14* exons, *Kb* kilobase. Variants IVS9-56G>A** and IVS9-17G>A** were published as mutations, but (Vilboux et al. 2009) reported that they are most likely benign variants. Mutations G115fs* and V157fs* are caused by genomic deletions which are predicted to cause exon 6 and 8 skipping, respectively, thus leading to frameshift and preliminary stop of translation (Zaťková 2011)

AKU-causing mutations are distributed throughout the entire HGD gene with a somewhat higher prevalence in exons 6, 8, 10 and 13 (Fig. 7.1). Missense mutations are the most numerous with 77/115 (66.37 %), followed by an equal number of small deletions and insertions causing frameshift and splicing mutations, each in 14/115 (12.2 %), and then nonsense mutations in 7/115 (6 %). The 23 most frequent mutations are present in 361 out of 496 (72.8 %) AKU chromosomes observed worldwide (Zaťková 2011). There are about 30 AKU chromosomes reported in which no HGD mutation was identified (Aquaron et al. 2009; Beltrán-Valero de Bernabé et al. 1998; Ladjouze-Rezig et al. 2006; Mannoni et al. 2004; Muller et al. 1999; Phornphutkul et al. 2002; Vilboux et al. 2009). These chromosomes might carry deep intronic mutations affecting splicing that could not have been identified when only exons with short neighbouring intronic parts were analysed in the

patients. They might carry mutations in the promotor region or in other cis regulatory sequences that also have not been captured using classic mutation detection methods. The most frequent mutation in Europe representing about 20 % of the mutations is Met368Val (replacement of methionine by valine at position 368), whilst in the Dominican Republic it is Cys120Trp and in Slovakia it is IVS2 + 1G → A. No typical pattern was found in the USA. It has to be noted that the majority of mutations are rare, and they are characteristic for a certain location or family (www.cib.csic.es/~akudb/index.htm). Beltrán-Valero de Bernabé et al. (1998) showed in 1998 that patients from different countries who shared the same mutations – M368V, V300G or P230S – also shared the same haplotype. Alternatively, haplotypes differed among these patients only in regions distal to the mutation position; thus, the differences could be explained by recombination events. The authors concluded these were most likely old mutations introduced to Europe with the founder populations, and they have spread throughout Western Europe along with different migrations. The "CCC" sequence motif and its inverted complement, "GGG", are preferentially mutated (Beltrán-Valero de Bernabé et al. 1999). Subsequently, nucleotide c.342 + 1 G was also described as a mutational hot spot in HGD (Zaťková et al. 2000a, b, c). Although most of the mutant enzymes showed a complete lack or very low levels of enzyme activity, five mutations led to specific activity that was 22–37 % of the wild type (E42A, Y62C, A122D, D153G and M368V). So far, no apparent correlation between the type of mutation and excretion of homogentisic acid in urine or severity of the disease has been found (Phornphutkul et al. 2002; Goicoechea et al. 2002; Uyguner et al. 2003; Ladjouze-Rezig et al. 2006; Zaťková 2011). As a consequence of the large number of mutations, patients with AKU can have two different mutations on a given locus, i.e. he/she is a heterozygote. As both alleles are not functional, such a condition is called compound heterozygous. It means that a patient with AKU is not always a recessive homozygote as it is understood according to the original classical conception of the genetic determination of this disease.

In Slovakia, within the rather relatively small population of 5.5 million, 208 patients have been registered (Sršeň et al. 2002) and a total of 12 different HGD mutations have been established, revealing a remarkable allele heterogeneity of AKU in this country. An allelic association was performed for 11 HGD intragenic polymorphisms in a total of 69 AKU chromosomes from 32 Slovak pedigrees. These were then compared to the HGD haplotypes of all AKU chromosomes carrying identical mutations characterized thus far in non-Slovak patients in order to study the possible origin of these mutations (Zaťková 2011). Based on the analysis and comparison of haplotypes, two groups of HGD mutations were observed in Slovakia. To the first group belong mutations P230S, V300G, S59fs (R58fs), M368V and IVS1-1 G>A, which account for 17.3 % of the Slovak AKU chromosomes and thus provide a marginal contribution to the AKU gene pool in this country. The most frequent European mutation, M368V, is present in one copy in only two unrelated Slovak families. Mutations of this group are shared by different populations and have most likely been introduced into Slovakia by the founder populations that spread throughout Europe (Zaťková et al. 2000a, b, c). The second group consists of the remaining seven

mutations established in 82.7 % of Slovak patients. These include the most prevalent G161R (44.2 %), D153fs (G152fs) (14.4 %), H371fs (P370fs) (11.5 %) and G270R (7.7 %), as well as IVS5+1 G>A present on three AKU chromosomes and the S47L and E178G mutations observed each in only one patient. It is likely that mutations from this second group originated in Slovakia. The distribution of the identified mutations within Slovak territory is also interesting. As previously reported, examination of the geographical origin of Slovak AKU mutations shows remarkable clustering in a small area in northwest Slovakia, with these mutations most likely originating in this area and spreading into other regions after the breakdown of genetic isolates in the 1950s (Zaťková et al. 2000a, b, c). As the combined sequence and haplotype analysis shows, 7 of the 12 AKU mutations (58.3 %) that most likely originated in Slovakia are associated with hypermutated sequences in the HGD, whilst worldwide it is 40/115 (34.8 %). Therefore, it is possible that an increased mutation rate in the HGD gene in a small geographical region is responsible for the high genetic heterogeneity in Slovak AKU (Zaťková et al. 2000a, b, c). However, it remains unclear which mechanism acted specifically on the HGD gene to increase its mutation rate, since similar targets are also present in other genes without evident elevated gene frequency in Slovakia (Sršeň et al. 2002; Zaťková et al. 2000a, b, c). The increased number of mutations could also be the result of random accumulation of mutations in the region. It has been suggested that the Valachian colonization during the fourteenth to seventeenth centuries may also have played a role in the increased prevalence of AKU in Slovakia (Sršeň et al. 2002; Zaťková et al. 2000a, b, c). The preservation of the most prevalent AKU variants, which either arose in Slovakia or were brought there, may be the result of a founder effect and genetic drift, due to the geographic isolation of villages in northwest Slovakia.

AKU belongs to rare diseases from the point of population genetics. Only slightly over 1,000 cases have been reported in the literature. The worldwide prevalence of AKU is not precisely known; it is estimated to be 1:250,000–1,000,000 (Beighton et al. 1993). However, it has got wide ethnic and geographic distribution. Its occurrence has been reported in various populations in Europe, America and Asia, i.e. this disease is not restricted to a certain race, but it affects individuals of various ethnic groups. It is interesting that AKU occurrence was not observed in the Gypsy ethnic group in Slovakia. Findings of typical ochronotic spondylosis in Egyptian mummies dated about 1500 B.C. suggest its occurrence in the ancient past (Beighton et al. 1993). The highest worldwide incidence of AKU is found in the Dominican Republic and Slovakia. For example, overall 149 patients were registered in Slovakia in the 1980s. A 6-year screening of the newborn population in Slovakia (417,122 newborns who survived the first 4 weeks of life) revealed about 1:19,000 incidence (Sršeň 1984). In certain regions of Slovakia, the disease incidence was up to ten times higher. These regions developed for a long time (until World War II) as genetic isolates, e.g. Kysuce, Orava and surroundings of Trenčín and Horehronie – the inbreeding coefficient (F) ranged from 0.003 to 0.029 (Sršeň 1984). The higher AKU incidence in these regions is probably a consequence of their long-term geographic isolation and endogamy, in addition to such genetic factors like genetic drift and founder effect. Break-up of isolates after World War II resulted in the gradual decrease of AKU incidence in Slovakia. On the basis of

genetic population analyses, the frequency of gene/allele for AKU in Slovakia was estimated to be 0.7 % – q=0.007 (Sršeň 1984). This value is relatively high, but in contrast to AKU frequency, it will not be significantly affected by break-up of isolates. On its basis, it is possible to perform certain genetic estimates that are not accurate with regard to several factors, which have to be taken into account in population analyses. However, they enable us to obtain several data of orientation value that can be useful from a practical point of view. On the basis of these estimates, there could be more than 250 patients with AKU in the whole current population of Slovakia (about 5.5 million citizens). The frequency of heterozygotes, i.e. healthy carriers of the pathological allele, would be approximately 14 persons per 1,000, and two matrimonies out of 10,000 would carry the risk of 25 % of two healthy heterozygotes having children with AKU. It has to be emphasized that in the case of consanguineous marriages or marriage of the people who come from the above-mentioned endogamous locations, the risk of AKU incidence in children is even higher. These data demonstrate that AKU problems and of course subsequent ochronosis and ochronotic arthropathy are topical in the Slovak population even nowadays and attention has to be paid to this disease.

With regard to the aforementioned frequent incidence of AKU in the Slovak population, it has been the subject of long-term research. Four institutions in Slovakia were devoted to its study – National Institute of Rheumatic Diseases in Piestany, Institute of Clinical Genetics of Jessenius Medical Faculty of Comenius University in Martin, Institute of Molecular Physiology and Genetics of Slovak Academy of Sciences in Bratislava and Faculty of Natural Sciences of Comenius University in Bratislava. The following years are important from the historical point of view:

- 1947 – The first reported case of AKU and ochronosis in Slovak population (Siťaj 1947).
- 1956 – The first monograph on AKU in world literature was published in Slovakia (Siťaj et al. 1956).
- 1950–1960 – There were 182 cases of AKU found in Slovak population (four million citizens). The patients came from 28 families. It was the largest group of patients worldwide (Červeňanský et al. 1959).
- 1966 – Hüttl et al. described inclusions of ochronotic pigment in the cytoplasm of synovial fluid cells (Hüttl et al. 1966).
- 1974–1984 – Large population studies were performed in Slovakia. Their results are summarized in the second monograph on AKU (Sršeň 1984).
- 1980–1981 – An analysis of 90 families with AKU occurrence confirmed an autosomal recessive type of inheritance in Slovak population (Kaprálik and Sršeň 1980).
- 1993–1994 – AKU gene was localized on the long arm of chromosome 3 in humans (3q). Slovak authors also contributed to this discovery (Janocha et al. 1994).
- 1994–2000 – Molecular characterization of mutations in HGD gene in Slovak population (Zaťková et al. 1999, 2000a, b, c).

Advances in the field of AKU molecular genetics contributed not only to a better understanding of the disease etiopathogenesis, but they were also significant from a

practical point of view as they enabled disease diagnostics at DNA (gene) level which gives a high-quality definitive diagnosis. DNA analysis can be used for identifying healthy carriers of the pathological allele (heterozygotes), which was not possible by biochemical methods. This knowledge can be used in genetic counselling. Knowledge of the molecular-genetic basis of AKU is very promising for the development of new approaches to the therapy of this disease which is currently aimed at symptomatic treatment of some complications and is not always effective. Two options of causal therapy are possibilities – substitute administration of HGD enzyme and gene therapy at the level of the somatic cell. Direct administration of the enzyme faces several problems, especially its localization in the liver, but also the route, the dose and the period and interval of administration. The most convenient option would be the application of the so-called recombinant enzyme. An example of such replacement enzymatic therapy is Gaucher's disease. Somatic gene therapy currently moves from animal studies to clinical trials in humans, e.g. in phenylketonuria. The normal functional gene is given to the patients using a suitable vector. According to current knowledge and experience with gene therapy, it is sufficient to achieve 5 % expressivity of the gene in order to yield its curative effect. Such an approach is supported by the recent results of a liver transplantation in an AKU patient in whom the progression of clinical and radiological changes was stopped, because hepatocytes of the transplanted liver had a functional HGD gene (Kobak et al. 2005). Gene therapy is a great hope for AKU patients because we still cannot treat this disease, which often causes disability and considerable suffering. Several other treatment strategies have also been suggested for AKU including nitisinone, the triketone herbicide, which inhibits the 4-hydroxyphenylpyruvate dioxygenase enzyme that produces HGA (Suwannarat et al. 2005).

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Experimental Alkaptonuria in Animals

8

James A. Gallagher

Content

Throughout the history of research into alkaptonuria (AKU), there have been many attempts to develop animal models to study the disorder. Several authors have fed experimental animals with diets high in phenylalanine or tyrosine and detected homogentisic acid in the urine (Papageorge and Lewis 1938; Butts et al. 1938; Abbott and Salmon 1943). In some of these studies, it was reported that dietary phenylalanine was more effective than a comparative amount of tyrosine in inducing alkaptonuria, and this effect has never been completely resolved. Despite the elevated urinary levels of homogentisic acid achieved in these animals, no evidence for the deposition of ochronotic pigment in tissues was reported in any of these studies.

The first evidence for the induction of ochronosis in experimental animals was derived from studies in which homogentisic acid was administered directly to animals (Moran and Yunis 1962). Multiple injections of homogentisic acid into the knee joints of rabbits led to pigmentation of the articular cartilage and synovium. These researchers also detected pigment in adjacent tissues including tendon and muscle. In some cases, the animal went on to develop severe arthropathies. It is interesting to note that delivery of HGA by intravenous or intraperitoneal injection failed to induce ochronosis.

The advent of techniques to induce genetic changes in experimental animals has led to major advances in developing animal models of disease. A mouse model of alkaptonuria was first developed at the Pasteur Institute in 1994 through a

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mutagenesis programme using N-ethyl N-nitrosourea (ENU) (Montagutelli et al. 1994). These researchers identified a mouse strain with a recessive mutation that mapped to mouse Chr 16 close to the D16Mit4 locus that is homologous to human 3q. The mice have a truncated HGD protein resulting from a splice mutation in the HGD gene. Mice homozygous for the mutation show high levels of urinary homogentisic acid, which causes darkening of their bedding. Other than the presence of HGA in the urine, no other pathology was detected, and initially it was thought that these animals did not exhibit ochronosis. Histological studies showed no signs of pigmentation in any of the tissues examined, which included the knee, hip, ankle, spine, liver, kidneys and tail, and there was no evidence of arthritis.

Several theories were advanced to explain the lack of ochronosis in these Hgd–/– mice. Unlike humans, mice have endogenous production of vitamin C, which some authors have suggested might be protective against ochronosis. It has been also postulated that the absence of observed pigmentation might be due to lower circulating HGA as a result of more efficient excretion of HGA, the shorter lifespan of the rodent or by lighter static loading of joints because of their quadrapedal mode. However, the recent studies described below indicate that lack of detection of ochronotic pigment was related to the sensitivity of the techniques and that Hgd–/– mice are susceptible to ochronosis.

The first reported detection of pigmentation in AKU mice was an anecdotal observation made by Fernandez-Canon while working in the group of Grompe in Portland on a complex double knockout mouse model. They were investigating tyrosinaemia type 1 (HT1), the most common genetic disease of tyrosine metabolism, which is caused by a lack of fumarylacetocacete acid hydrolase (FAH). They combined their HT1 mouse model (Fah–/–) with AKU heterozygosity (Hgd+/–). For survival, these mice required continuous administration of nitisinone to prevent the accumulation of fumarylacetoacetate and other metabolites with hepatorenal toxicity. They observed that in a small percentage of the mice when nitisinone was withdrawn, the mutagenic environment caused some hepatocytes to undergo a loss of heterozygosity at the Hgd locus. This resulted in the generation of Hgd –/– Fah–/– hepatocytes, which had a selective advantage that allowed clonal expansion, eventually repopulating the liver. These mice consequently acquired an alkaptonuric phenotype, which effectively rescued them from HT1. Evidence of pigmentation was detected serendipitously in some of these mice, and this provided the stimulation to undertake a systematic study in which the presence of ochronosis was confirmed (Taylor et al. 2012). The detection of pigmentation was facilitated by the application of Schmorl's stain which considerably improves the sensitivity of pigment detection (Tinti et al. 2010). It is likely that the ochronosis observed in these mice is in part accelerated by the underlying renal pathology. Reversion to the AKU phenotype in the Fah–/–, Hgd+/– mice is not predictable, and thus they do not provide a practical experimental model of AKU and ochronosis. However, these findings did provide a significant breakthrough, demonstrating that mice are susceptible to ochronosis. Furthermore, the earliest signs of pigmentation observed in these mice are in the chondrocytes in the calcified cartilage layer which reflects the initiation and progression of ochronosis in human AKU (Taylor et al. 2012).

In order to develop a consistent mouse model of AKU with no confounding pathology, a research programme was established at the University of Liverpool, UK. Four Hgd−/− mouse breeding pairs on a BALB/c background strain were obtained from Dr X Montagutelli (Pasteur Institute, France), and a colony was established. Study of the natural history of AKU mice revealed that the mean plasma HGA concentration across the mouse lifetime was 0.148 ± 0.019 mM (mean ± SE), with no obvious increase or decrease with age, despite significant variation between individuals. This was three- to fourfold higher than published values for AKU in humans which in one report were 0.039 ± 0.015 mM (mean ± SE). These data demonstrate that the apparent lack of ochronosis in the AKU mouse was not related to more efficient renal clearance of HGA or lower circulating levels.

The initial strategy to induce ochronosis in the AKU mice was to investigate whether physiological challenges might induce pigmentation, including preventing endogenous production of vitamin C by cross-breeding with gulo-knockout mice, impeding renal function or stimulating joint loading through neuromuscular stimulation. However, careful histological observation of knee joints coupled with the use of Schmorl's stain to enhance the sensitivity of detection revealed that AKU mice undergo spontaneous ochronotic pigmentation without any physiological or pathological stimulation. Chondrocyte pigmentation was first detected at 15 weeks old in the femoral and tibial calcified cartilage, and there was an approximate linear progression in the degree of pigmentation with age (Preston et al. 2014). The initial pigmentation was restricted to the pericellular matrix surrounding individual chondrocytes in the calcified cartilage, with no cellular staining present. Later pigmentation of both chondrocytes and pericellular matrix could be observed. Pigmented cells could not be observed without pigmented matrix, indicating that ochronosis is initiated in the pericellular matrix. By 65 weeks, ochronosis was extensive throughout the femoral and tibial articular cartilage but, unlike human AKU, was restricted to the calcified zone. Pigmented chondrocytes frequently appeared pyknotic, sometimes with pyknotic and viable chondrocytes appearing in the same isogenous group of cells. Older AKU mice began to show signs of osteoarthritis, including cartilage loss and osteophytes and changes to the subchondral bone. However, wild-type mice are also prone to osteoarthritic changes, and the rate and severity of joint disease was only slightly more evident in AKU mice. Apart from the ochronosis, the AKU mice appeared to be healthy; they grew well and had normal levels of activity.

Subsequently a study was undertaken on the effect of nitisinone on plasma HGA and ochronosis, which was quantified by counting pigmented chondrons in sections of knee joints. Lifetime treatment with nitisinone provided in drinking water resulted in an 88 % reduction in plasma HGA and complete prevention of ochronosis. Nitisinone was well tolerated over the mouse lifetime, with no noticeable changes in health, growth rate or behaviour between the control and treated groups. Furthermore, it was established that, if treatment with nitisinone was commenced after 35 weeks by which time the mice had extensive pigmentation, although the drug prevented further ochronosis it did not reverse it.

In summary, throughout the history of AKU research, there have been many attempts to develop experimental models of ochronosis. Recent studies on the AKU mouse, originally identified by Montagutelli and co-workers, have revealed that this an excellent disease model to investigate the initiation and early progression of ochronosis and to investigate the efficacy of therapeutic strategies. This model has been used to confirm that nitisinone is completely effective in inhibiting ochronosis. This model will undoubtedly be a major experimental resource in identifying and screening new therapies for AKU including gene repair and replacement.

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Clinical Manifestation of Alkaptonuria and Ochronosis

9

Jozef Rovenský and Tibor Urbánek

Content

In the clinical picture of genuine human alkaptonuria, excretion of homogentisic acid in urine remains the only pathological sign for decades. Meanwhile in the body of the patients with alkaptonuria, the process takes place that is much more serious from pathological-anatomical as well clinical point of view – deposition of homogentisic acid and its derivatives in some tissues causes their discoloration. According to Bürger (1954), vital staining of so-called bradytrophic tissues takes place. Basically it is a benign process that is asymptomatic for a long time. As time goes by, age and lower resistance of tissues prepare the conditions for pathological changes, namely, of degenerative character, with the subsequent manifestation of articular as well as extra articular signs. Tissues containing homogentisic acid and its oxidative polymer – pigment – have typical yellow-brown or even black colour. The first pathological-anatomical description of this disease was made by Virchow in 1865 (Virchow 1866).

During the autopsy of a 65-year-old patient who died of heart aneurysm with the signs of circulation insufficiency and overall exhaustion, Virchow accidentally found special dark colouration of cartilages of ribs, several joints, pelvic

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synchondroses, larynx, nose and ears. Vascular intima was similarly coloured. The aforementioned tissues were 'black as ink', like they had been submerged into common ink. All affected tissues were pathologically changed; cartilages showed 'signs of progressive overgrowth' (Umber 1925). Virchow assumed that the substance contained in tissues was a derivative of haematin. Therefore, he named this pathological condition as 'ochronosis' (ώχρός = yellow, νόσος – disease). Marshall (1887) found that the chemical principle of this metabolic disease is excessive excretion of homogentisic acid. Later on, Albrecht and Zdarek (Albrecht 1902) anticipated that the cause of ochronosis is alkaptonuria. More detailed analysis of ochronosis development was performed by Pick (1906a, b) who had the opportunity to observe pathological condition in chronic carbol poisoning supplied to the body for a prolonged time by compresses and washing of ulcers. He found out that ochronotic changes of tissues in alkaptonuria and carbol poisoning were identical, and he overturned Virchow's theory that ochronotic pigment came from haematin. He assumed that pigment formation in ochronosis represents a certain form of melanin formation and that it is formed by means of enzyme tyrosinase from hydroxylated derivatives of benzole like hydroquinone, benzcatechin as well as homogentisic acid. With regard to the fact if ochronosis is caused by a substance artificially brought to the body (carbol poisoning) or if it is caused by metabolic disorder (inability to degrade homogentisic acid), Pick recognises exogenous and endogenous ochronosis (Pick 1906a, b). In more recent papers, it is noted that in a majority of cases endogenous ochronosis arises in alkaptonuria and only rarely in combination of alkaptonuria with melanuria (Wooley 1952). Exogenous ochronosis develops in case of phenol, pyrocatechin and hydroquinone intoxication. The clinical picture of exogenous and endogenous ochronosis is very similar; however, there is a substantial difference in urine findings (Wooley 1952). Exogenous ochronosis caused by carbol is very rare, because treatment of crural ulcer with this substance was withdrawn.

Kleinschmidt (1922) performed chemical analysis of ochronotic pigment. He found out that it did not contain iron, and it did not stain fat. Its colour does not become more intense after the application of silver nitrate according to levaditis method, and it becomes white after the application of hydrogen peroxide. It is poorly soluble in hydrochloric acid and well soluble in alkaline substances. In technical staining and chemical analysis, it seems to be similar to melanins.

More recent works on pigment formation confirm Pick's assumption that ochronotic pigment forms by oxidation and polymerisation of homogentisic acid. This reaction is probably catalysed by enzyme tyrosinase. Pigment polymers can arise from various polyhydroxyphenyl and aminophenyl compounds with the groups in o-position and p-position. Melanins (Lerner 1953; Keller et al. 2005) include polymers arisen from various substances; amino acids phenylalanine, tyrosine and tryptophan usually participate in their formation. Their name is sometimes based on the name of substance they come from, e.g. dopa-melanin, adrenalin-melanin, homogentisic acid-melanin, p-phenyldiamine-melanin, etc. Natural melanins are usually bound to proteins. They can change as a reversible oxido-reduction system, while the oxidised form is black and the reduced one is brown. Melanin granules are

displayed as characteristic regular spheroid particles in electron microscopy. No microscopic differences were found among various types of melanins. Several factors participate in melanin formation: hormonal, enzymatic, physical-chemical, etc. The product of intermediate lobe of pituitary gland melanocyte-stimulating hormone (MSH) is the hormonal factor. Among the other factors concentration of tyrosine, tyrosinase and molecular oxygen, temperature, concentration of hydrogen ions, oxido-reduction potential, copper presence, etc., are important. In vitro, ochronosis could be induced by inserting the pieces of cartilage to the solution of homogentisic acid (Gross and Allard 1907). The cartilage successively became more and more dark, acquiring black colour, and it did not differ from ochronotic cartilage obtained from pathological-anatomical specimen in any aspect. Katsch (1932) proved that all tissues are able to produce ochronotic substances with enzymes acting on homogentisic acid.

Authors who had the opportunity to observe rare cases of alkaptonuric ochronosis in autopsies found the changes on nearly all organs. Changes on cardiovascular organs were particularly marked: patchy pigmentations on pericardium (Fisher and Davis 2004; Lichtenstein and Kaplan 1954) and dark brown colouration of endocardium and vascular intima due to impregnation by ochronotic pigment (Galdston et al. 1952; Umber 1925; Gaines and Pai 1987). Sclerotic changes and calcifications are detectable on aortic intima, valvular endocardium and tendon chords. Coronary arteries are sclerotic, but without pigmentations (Galdston et al. 1952). Some authors found pigment deposition in stratum musculare of blood vessels (Švejda 1945). In the lungs, ochronotic changes can be found in bronchial cartilage and around bronchi. Pigment deposition can be detected in the kidneys, suprarenal glands, thyroid gland, testicles and especially in epididymis, less in spleen and pancreas. The pigment is deposited in the form of granules, especially around blood vessels, less in parenchymal cells and sometimes in stroma. Small brown concrements were reported in the prostate (Švejda 1945).

Clinical symptoms of involvement of visceral organs in our patients and in those with anticipated advanced pathological-anatomical changes seem to be relatively mild. In the cardiovascular system, careful physical, X-ray and ECG examinations revealed changes only suggesting aortic sclerosis. Other clinical symptoms did not exert significant abnormalities, and sclerotic changes appeared to be adequate to age. Our observation is in accordance with the literature. Myocardial infarction was attributed to ochronotic changes only sporadically (Lichtenstein and Kaplan 1954; Coodley and Greco 1950). Involvement of other visceral organs could not be revealed by physical or laboratory examination, and the only pathological laboratory finding was alkaptonuria. When assessing medical history from the point of visceral organ symptomatology, we found calculosis relatively frequently. Three out of our 8 female patients with alkaptonuric ochronosis had gall bladder stones, and 2 patients out of our 19 male patients suffered from kidney stones.

We can observe characteristic changes on skin and nails in advanced stages of alkaptonuric ochronosis. Skin has a special grey-brown colouration that is most pronounced on areas exposed to sunlight. Punctiform black pigmentations are typical on face, namely, around eyes. Skin has a blue-green colouration in armpits.

Histological analysis of skin affected by ochronosis found that delicate pigment granules are not located in the cells of epidermis and cutis but in vascular intima and in spread macrophages (Lichtenstein and Kaplan 1954). Nails have a blue-like tinge, and there are brownish stripes in distal parts. Hardness and fragility of nails is marked.

Homogentisic acid was also found in sebaceous glands, a finding confirmed by chromatography in our patients. Umber (1925) and Kocyigit et al. (1998) did not find homogentisic acid in sweat even after thickening, and they assume that sebaceous secretion was present in positive cases. In our patients, we also did not detect homogentisic acid in sweat either by chromatography or reaction with a-a-dipyridyl, although the sensitivity of these assays is very high (see the description of methods for identification of homogentisic acid). In general, literary sources present that in alkaptonuria, ochronosis develops alter several decades. Umber (1925) states that all his patients were affected by ochronosis at the age of 40–50 years. In more detailed analysis of this issue in our patients, we found out that clinical manifestations of ochronosis occurred at a younger age. The first articular signs occur between the age of 30 and 40 (average age: 34.6 years). The exact onset of auricle colouration cannot be determined because many patients do not know about it and often it is found accidentally during examinations in older age. However, it seems that auricle colouration precedes clinical symptoms of ochronotic arthropathy. In two our patients, colouration occurred at the age of 17–18 years, while the first X-ray changes on joints could be observed at the age of about 30 years. Probably the earliest clinical manifestation of alkaptonuric ochronosis is eye pigmentation. The average onset of changes around eyes is even harder to determine than in the auricles as the initial pigmentations are very discrete and they can be detected only by experts. In one of our patients, ochronotic pigmentations on sclera were confirmed during an ophthalmologic examination at the age of 8 years (see the chapter on eye changes).

Many authors dealt with the issue of transition of alkaptonuria to ochronosis, but they could not obtain significant results from sporadic observations. Umber (1925) stated that ‘not every patient with alkaptonuria will live until the development of ochronosis’, but on the other hand, he presented the fact that in a family followed by him, all individuals affected by alkaptonuria had the same marked clinical signs of alkaptonuric ochronosis at the age of 40–50 years. According to some other authors, alkaptonuria leads to ochronosis in approximately one half of the cases (Kocyigit et al. 1998). We analysed this issue in detail in our patients, and we found out the results given in Table 9.1.

As it can be seen in Table 9.1, out of 21 alkaptonuric patients diagnosed in the first decade, ochronosis was detected only in one of them (eye involvement), i.e. 4.7 %. In the second decade, we did not find any ochronotic patient; in the third, fourth and fifth decades, the percentage of ochronosis markedly increased reaching the highest value of 62.5 %, and later on in the sixth, seventh and eighth decades, it gradually decreased to the value of 25 %. If any conclusions can be made from this material, it could be deduced that the critical age for ochronosis development is the fifth decade and in alkaptonuric patients passing to elder age groups, ochronosis does not occur or tissue pigmentation is so limited that it is not clinically

Table 9.1 Incidence of alkaptonuria and ochronosis in individual decades and the percentage of alkaptonuria cases associated with ochronosis

Decade	Total number of patients with alkaptonuria and ochronosis	Number of patients with ochronosis	Percentage of cases of alkaptonuria associated with ochronosis
I	21	1	4.76
II	14	0	0
III	12	1	8.3
IV	18	9	50.0
V	8	5	62.5
VI	14	8	57.1
VII	5	2	40.0
VIII	4	1	25.0

manifested. Microscopic or discrete changes detectable by histochemical methods cannot be ruled out in these cases of alkaptonuria.

As it was mentioned before, deposition of homogentisic acid and its polymer – ochronotic pigment –results not only in vital staining of so-called bradytrophic tissues but also in large regressive changes that are particularly marked in articular cartilaginous and periarticular tissues (tendons).

Boedecker (1859) was the first one to point out the relationship between alkaptonuria, ochronosis and articular signs. Gross and Allard (1907) called these changes improperly as 'alkaptonuric arthritis'. In ochronotic arthropathy, all vitally important articular structures are affected. Procházka and Hněvkovský (1953) even found the impregnation in subchondral layers of the joints. Vascular system nourishing the bone ends up in this zone, and lymphatic sinuses take over the nutrients supply. The authors hypothesised that the principal injury of cartilaginous tissue in ochronosis is the blockade of lymphatic sinuses resulting in change of concentration of hydrogen ions. These layers, where the bone neogenesis takes place, are extraordinarily sensitive to pH changes. The support for this concept is the presence of ochronotic changes on vascular intima. The changes are most pronounced in terminal arterioles, thus affecting local metabolic processes. We also have to assume successive exposure of other layers of cartilage to homogentisic acid and its more or less polymerised derivatives (see Chap. 27 the 'Case Report').

Homogentisic acid causes the loss of cartilage elasticity and its successive hardening. More recent histochemical observations demonstrate that proteoglycans together with their glycosaminoglycans (previously called mucopolysaccharides) that bind a large amount of water with their negative charge are responsible for cartilage elasticity (Knudson and Knudson 2001). Apparent loss of proteoglycans can be seen in degenerative changes of articular cartilage. It is anticipated that homogentisic acid and its polymers disrupt the matrix of connective tissue by unfavourable impact on proteoglycans (mucopolysaccharide complex of the matrix). However, it seems that ochronotic injury is not caused by shredding of superficial layers to fibres as seen in cartilages affected by arthrosis, but it probably causes its

glassiness and fragility. Cartilage can easily fragment to small pieces that are often similar to pins with the development of so-called chondrosis dissecans. Besides these changes, disorders of cartilage continuity and cracks reaching to bone with various depths occur. Secondary changes in the bone similar to arthrosis arise on this basis.

Small, sharp and hard fragments of cartilage affected by ochronosis accumulate in articular fissure, especially in so-called 'silent' places (recessus posteriores art. genus). They also penetrate to synovial membrane, and by irritation, they cause hyperaemia, increased secretion of synovial fluid and rarely granulation changes. Some authors call this synovial reaction as synovitis chondrodetritica, and they consider it a common manifestation of articular diseases in which increased wear and fragmentation of cartilage take place (chondromalacia patellae, osteochondritis dissecans and other conditions) (Hulten and Gellerstedt 1941; Lagier and Steiger 1980). Cartilaginous changes in the course of ochronotic arthropathy were considered by classical authors as the most serious and primary ones. The experience of Červeňanský et al. (1959) that is described in detail in analysis of histological changes suggests that the targets of ochronotic changes are tendons and vascular tissues. In spite of the fact that transient irritational synovial reactions take place in advanced stages of ochronosis, the basis of pathological articular manifestations are degenerative and regressive bone-cartilaginous changes.

Clinical manifestations of ochronosis in the joints, eyes and ears are presented in the next chapters.

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Ochronotic Arthropathy

10

Jozef Rovenský and Tibor Urbánek

Content

We had the opportunity to observe the clinical course of ochronotic arthropathy in the group of 26 patients suffering from this disease. As we followed some of the patients for the period of up to 10 years, this observation period enabled us to catch individual stages of disease development from initial symptoms to final, often disabling stages. We also had the opportunity to catch the dynamics of the whole comprehensive pathological process and to compare the development of articular pathological changes of ochronotic arthropathy with other similar pathological conditions, especially generalised osteoarthrosis.

We present the group of 26 patients with clinical manifestation of the disease in various stages of arthropathy. The average age of our patients was 47.8 years (18 males and 8 females). The average duration of joint problems was 12.65 years – 1 year least, 34 years most. The first pathological symptoms on joints occurred at the age of 34.4 years in average – 27 years soonest and 51 years latest. The ratio of men to women in our group of patients was 2:1. Hogben et al. (1932) and Phornphutkul (2002) who present the summary of sporadic published cases also found such a ratio of men to women.

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The issue of initial symptoms of ochronotic arthropathy has not been discussed in literature yet, as sporadic papers usually aimed at other than rheumatologic problems discuss only the advanced stages of the disease. We followed several patients with initial articular problems, and we found out that the first signs of ochronotic arthropathy appeared at a relatively young age. We observed the disease in our six patients already short time after arthropathy onset, while we examined subjective problems of the patients as well as objective clinical and X-ray symptoms. The average age of these patients at disease onset was 29.3 years. It is interesting and surprising that the later the ochronotic arthropathy was manifested, the faster it progressed and induced serious changes in joints.

Before we describe arthrotic finding of ochronotic arthropathy, we will present some of the general knowledge gained by serial examinations of our patients. As our patients have lower height in general and they report gradual height reduction, we analysed this issue in more detail. We found that the average height of our 26 patients with ochronotic arthropathy was 158.5 cm in men and 151 cm in women, i.e. men are 12.9 cm lower and women 6.1 cm lower than the average height of our population. Their height successively decreases with the disease progression. The most pronounced height reduction occurred in a patient whose height at disease onset confirmed by an official document was 163 cm and on admission to our institute, after 20-year duration of the disease, his height was 8 cm less when compared to initial value. By the precise anthropometric measurement of our patients (Table 10.1), we found that spine contributed most to the height reduction. This spine shortening can be explained by narrowing of intervertebral spaces on the basis of disc regressive changes, while flattening of vertebrae also participates in height reduction. Our observation is confirmed by finding of Bürger and Schulze (1953) who found that 6 vertebrae of ochronotic spine correspond in the length to 5 vertebrae of normal spine.

Table 10.1 Anthropometric measurements of patients with ochronotic arthropathy compared with average values of our population

Anthropometric measurements	Body height		Height in sitting position		Acromion-dactylion	Trochanterion
Gender	Males	Females	Males	Females	Males	Females
Values in healthy people (cm)	171.4	157.1	90.6	83.9	75.5	84.2
Values in patients with ochronotic arthropathy	158.5	151.0	81.2	77.2	77.5	83.7
Value differences	12.9	6.1	9.4	6.7	−2.0	0.5

Average values of anthropometric measurements were obtained from population survey by Associate Professor RNDr. V. Fetter, Head of Anthropologic Institute in Prague and his co-workers RNDr. J. Suchý and RNDr. M. Prokopec (Fetter et al. 1967)

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Clinical Manifestation of Ochronotic Arthropathy in Spine

11

Jozef Rovenský and Tibor Urbánek

Content

The predominant clinical presentation of ochronotic arthropathy in the skeletal system is the spine, which was affected by the disease in all our patients. While observing the patients in initial stages of the disease as well as during more detailed analysis of medical history, we found out that except for a few cases, the first subjective and objective signs were localised in spine area.

The patients complain of uncertain feeling of stiffness in lumbosacral area as the first pathological symptom that can be associated with mild pain. These sensations are more pronounced if the patient stays longer in unusual position, e.g. in anteflexion during work with the subsequent back straightening. Some patients also feel a certain block while back straightening that can be overcome only with pain. This stiffness is later accompanied with significant pain that is never as severe as in inflammatory diseases of spine. Several patients complain of undefined feeling of coldness in sacral area as the initial symptom of the disease.

Slow gradation of patients' problems is typical for ochronotic arthropathy; mild pain remains permanently localised and limited to lumbar area without irradiation into surrounding areas and without periodic fluctuations in its strength.

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Spine pain preserves its static character even during further progression of the disease and does not appear to intensify proportionally with the development and progression of pathological changes in spine. In some cases, ankylosing changes of the spine that cause its complete stiffness are accompanied only with minimal pain. This nearly painless stiffness of the spine is the phenomenon that can also be encountered in inactive stages of ankylosing spondylitis (AS), ankylosing hyperostosis, tabic arthropathy as well as healed specific or other processes in spine. It seems that disproportion between mild pain and progressive limitation of movement of individual spinal segments as well as described onset and initial development of the disease is typical for ochronotic arthropathy. However, we also observed some deviations. In one patient, the pain was so accentuated at the disease onset that it disturbed his sleep. In other four patients, a sudden episodic radicular pain suggesting acute lumbosciatic syndrome occurred during initial problems. In rare cases, while analysing medical history, we found an almost painless onset of spine stiffness.

Pain in ochronotic arthropathy has many common features with arthrotic pain. It is usually associated with movement and appears particularly during movement activation, while it regresses at rest. Recurrent micro-injuries deteriorate it. It definitely does not have inflammatory character. It is not markedly manifested at rest, and it is spontaneous only rarely. It is a dull pain and does not have crepitant, drilling and lancinating character as inflammatory pain.

Mild pain deterioration is basically always associated with higher physical activity. In our patients, we observed spontaneous exacerbations only very rarely, especially nocturnal paroxysmal pain that is so typical for AS. The pain reacts more considerably to mechanical rather than micro-climatic effects. Except for micro-injury and excessive strain, we did not find any exacerbating role of infectious, hormonal or peristasis in the development of pathological signs in joints. Arthropathy occurs without prodromes, and we also do not observe general symptoms typical for inflammatory process like weakness, weight loss, subfebrilities, higher erythrocyte sedimentation rate, anaemia, etc.

Examination of the patients reveals successive flattening of physiologic curves of the spine, even in the stage when X-ray findings are completely negative (Fig. 11.1). Unfolding of the spine is still normal in these signs, only later we observe decreased depression in retroflexion. Four of our patients we have followed for several years have got ochronotic changes on ears and mild subjective problems in spine area with incipient above-mentioned configuration changes and no skiagraphic changes detectable yet. In the next stage of the disease, we observe insufficient alignment of lumbar lordosis during forward bending. In some of the patients, we observed small irregular depressions in lumbar or lower thoracic spine comprising two to three spinous processes that did not align in anteflexion, but we could not qualify it as sulcus dorsalis persistens typical for spine in Bechterew's disease.

In addition to flattening of physiologic curves in ochronotic arthropathy, more pronounced and larger rigidity of lumbar and later on of thoracic section of the spine can be seen. Affected parts of the spine unfold still less and less in anteflexion, kyphotic arch becomes flatter and distal, and especially lumbar section remains in permanent extension position. Ochronotic spine gradually gets the shape that was

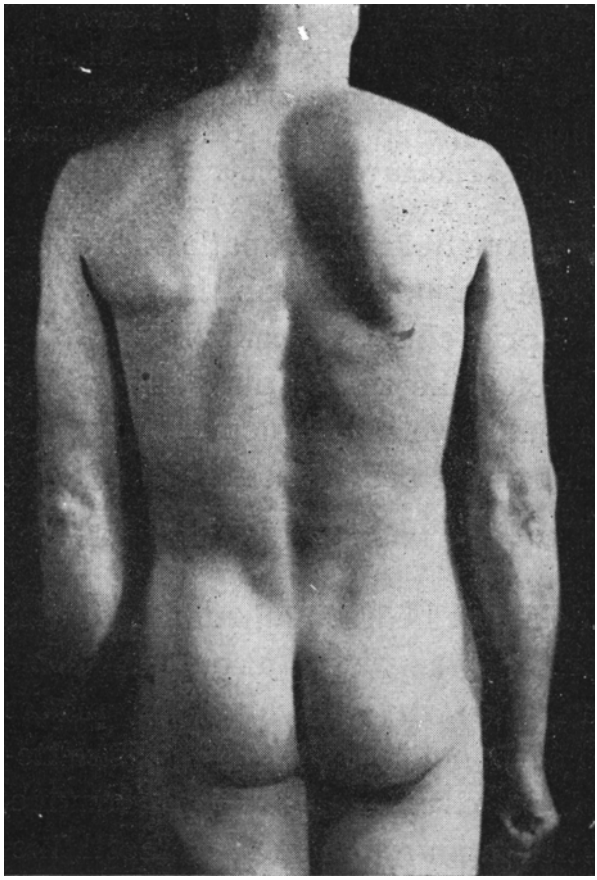

Fig. 11.1 Flattening of physiologic curves of the spine (thoracic kyphosis and lumbar lordosis). Initial stages of ochronotic changes in spine

described by Lenoch (1951) in AS type named 'spondylarthritis ankylopoetica cum columna vertebrarum extenta'.

We tried to assess grade of movement limitation of ochronotic spine. For this purpose, we chose several measurable signs that are used in Bechterew's disease (Lenoch 1941, 1951):

(a) *Stibor's distance*: During this measurement, we connect, in standing position, the spina iliaca dorsalis cranialis on both sides with horizontal line – dermograph. This line intersects L5 spinous process. From this point, we precisely measure the distance to vertebra prominens. In maximal anteflexion of a patient, this distance is elongated by the value called Stibor's distance. In normal humans, this distance is elongated by 9 cm at 170 cm height. This distance is longer in taller patients and shorter in smaller patients. Distance is longer in young people and shorter in elder ones. If the distance between proximal end of crista sacralis vertebralis (L5) and vertebra prominens is elongated by 9 cm, we say that Stibor's distance is +9. This distance numerically expresses the range of flexion movement of lumbar and thoracic spine.

(b) *Schober's sign*: The line between both spinae iliacae dorsales craniales is marked by a dermograph on the back of patients in standing position. This line intersects L5 spinous process. From this point, we measure, in standing position, 10 cm in proximal direction reaching LI spinous process. In healthy people, processes move away like a fan, the distance between L5 and LI is usually elongated by 5 cm, and this is called Schober's distance. This distance is significantly reduced in AS patients even in initial stages of the disease. In spondylarthrosis, this distance is elongated less than in healthy people. We designated this sign in abbreviated form as 'Schober × cm'.

(c) *Lateroflexion measurement*: The patient puts palms on thighs in standing position, and at the place where tip of middle finger touches the thigh, we will draw the line by dermograph. Then we will ask the patient to make a lateroflexion to the side and to move the hand on lateral part of the thigh to the most distal position. We also have to prevent the patient to simultaneously perform spine rotation in the course of lateroflexion. In maximum lateroflexion, we will mark the place on external lateral side of the leg where the tip of the middle finger can reach. Distance between both lines expresses the numerical motion performed by spine, hip joint and partially arm joint in frontal line. Under normal circumstances, a man can reach the plane, which intersects lower margin of patella with the tip of the middle finger. The distance between both lines should not be smaller than 20 in 170 cm high individual. We designated this sign in abbreviated form as 'lateroflexion × cm'.

(d) *Occiput-wall distance* (fleche of J. Forestier). A patient stands towards the wall in such a way so that heels, dorsal part of knees, calves, back and, if possible, the occiput touching it. The patient has to hold their head in such a position so that lateral angle of the eye and upper insertion of the helix would be on the same horizontal straight line. If the patient cannot touch the wall with the occiput, as it usually happens in ankylosing spondylitis, we will measure the horizontal distance between the most dorsal part of occipital bone and wall. This distance is called the occiput-wall distance or fleche. In our case report, we designated this sign as 'Forestier × cm'.

In this relatively initial stage of ochronotic arthropathy, we observe decreased mobility of the spine according to measurable values by about 50 %. The spine reaches this degree of involvement in 3–5 years.

During the further course of the disease, the flattened physiologic curves of the spine gradually form hardly noticeable arch with markedly large diameter with the apex at Th7–8 level. In this stage the whole thoracic and lumbar spine is rigid, measurable signs are reduced to 1/3–1/4 of original values. This condition develops in 6–8 years after the disease onset.

In completely developed clinical manifestation, lumbar and thoracic spine reaches total rigidity or even ankylosis. Measurable signs are reduced to minimal values. At the first sight, we can see that it is a severe ankylosing process of the spine that manifests with some features characteristic for ochronotic arthropathy. Flattening of physiologic curves in lumbar and thoracic spine reaches a high degree,

plasticity of spine exerts certain irregularities, some spinous processes stand out unevenly, and on the other hand there are depressions at some places as well as scoliotic curves limited to small sections (Fig. 11.2). Muscles are usually atrophic; spastic rigidity occurs very rarely.

We observed a deviation from the described spine flattening in our patient who had more pronounced thoracic kyphosis. We found out in his medical history that the patient had been working in a tannery where he had lifted 8–40 kg leathers from his youth. This one-sided load definitely had impact on spine formation.

In his cervical spine, we see certain discrepancy between X-ray and other clinical findings. In spite of relatively frequent skiagraphic changes, we observed significant movement limitation in this section only rarely, much less than in AS. When compared to osteoarthrosis, functional insufficiency of cervical spine in ochronotic arthropathy is definitely more pronounced. Limited mobility of cervical spine never reaches the degree observed in some of our patients. In these patients, ankylosing process affecting, namely, intervertebral discs expanded cranially as well as to the lower part of cervical spine, thus causing apparent small-arch hyperlordosis with

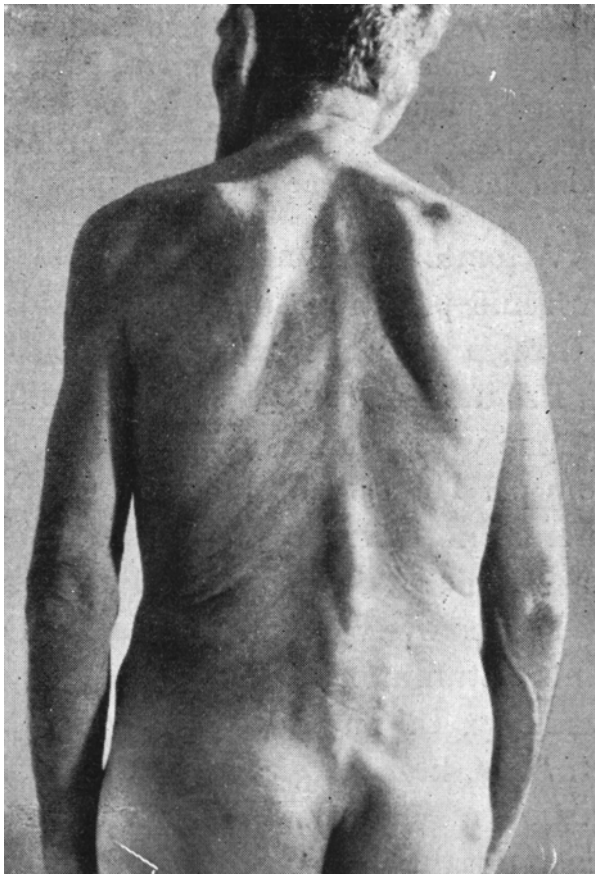

Fig. 11.2 Spine of the patient with advanced stage of ochronotic arthropathy

forward position of head in upper part of cervical spine. As the mobile part of cervical spine became shorter by about a half, the extent of head movements logically also became proportionately lower. Head lateroflexion was restricted most of all, and in one of the patients it was fixed at an angle of 15–20°. Rotational movements are limited, but mostly they remain preserved at an angle of a 20–25° angle. The head is fixed in forward projection, while bending backwards is limited (positive Forestier's sign also suggests it: 16 cm in one case and 19 cm in another case). However, the head is not tilted with the face to the ground as in AS type called spondylarthritis ankylopoetica cum kyphosi thoracali (Lenoch 1951), but it is in straight position that enables normal horizontal view and eye bulbi do not have to turn. Thus, Lenochs sign 'signum medici poplité flexi' is negative in ochronotic arthropathy. In a patient with ankylosing spondylitis, a physician has to bend patients' knees to examine oral cavity (Lenoch 1941).

This is again a substantial difference when compared with ankylosing spondylitis. It has to be pointed out that ochronotic arthropathy affects lumbar and lower thoracic spine in nearly the same extent and ochronotic changes of intervertebral discs have approximately the same intensity in both sections. Spondylarthrotic process also affects lumbar section most, but changes in thoracic section are much rarer. It can be assumed that there are certain differences in the mechanism of development of regressive changes in both pathological conditions. Some differences in spine shape also confirm it. While we observe tendency to kyphotic curves in arthrotic spine, we can see apparent flattening (dos plat) in ochronosis. There is a more pronounced irregularity of contours of spinous processes in ochronotic spine. The most marked difference between these two diseases consists in overall dynamics of the development of symptoms. Ochronotic arthropathy has more extensive and intensive progression, and it results much earlier and more severe rigidity of spine than osteoarthrosis. The difference between ochronotic arthropathy and AS is caused by pathological process itself. While in ochronotic arthropathy, a process of gradual calcification of intervertebral discs with associated degenerative changes takes place, progressive rigidity in AS is caused by inflammatory process in intervertebral junctions, in the area of small intervertebral and costovertebral joints with their obliteration. From the typical clinical signs that distinguish Bechterew's spine from ochronotic one, we present the most marked.

Nearly painless onset and progression of the disease is usual in ochronotic arthropathy, while in AS it is exceptional.

In ochronotic arthropathy, the contours of the spine successively disappear, but this is only observed in AS in minority of cases. The spine of healthy individuals has regular contours, and it forms single-arch contour on anteflexion, while sulcus dorsalis becomes straight and even vanishes (Fig. 11.3). In AS spine, contours are straightened, but sulcus dorsalis does not disappear even on maximal anteflexion (Fig. 11.4). In ochronotic arthropathy, irregularity of the contours comes to the fore. It is caused by prominent or at some other places depressed spinous processes. Sulcus dorsalis is not observed even in standing position, and plasticity of the spine basically does not change on anteflexion (Fig. 11.5). Chest respiratory excursions in AS are decreased or vanished, while they are preserved in ochronotic arthropathy.

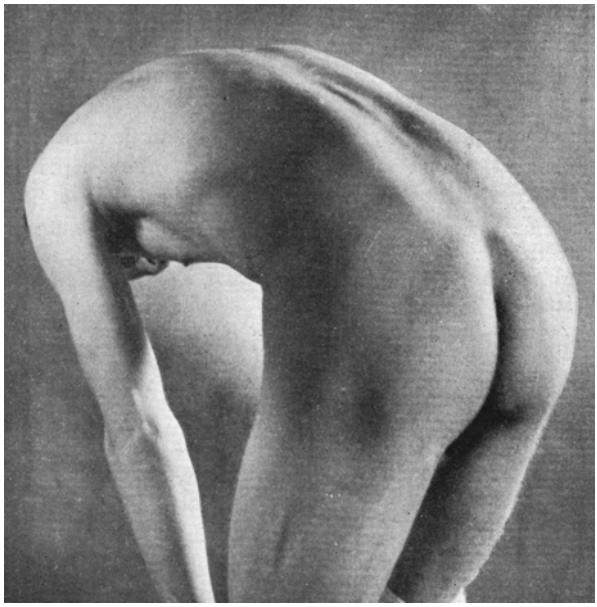

Fig. 11.3 Normal unfolding of the spine in anteflexion

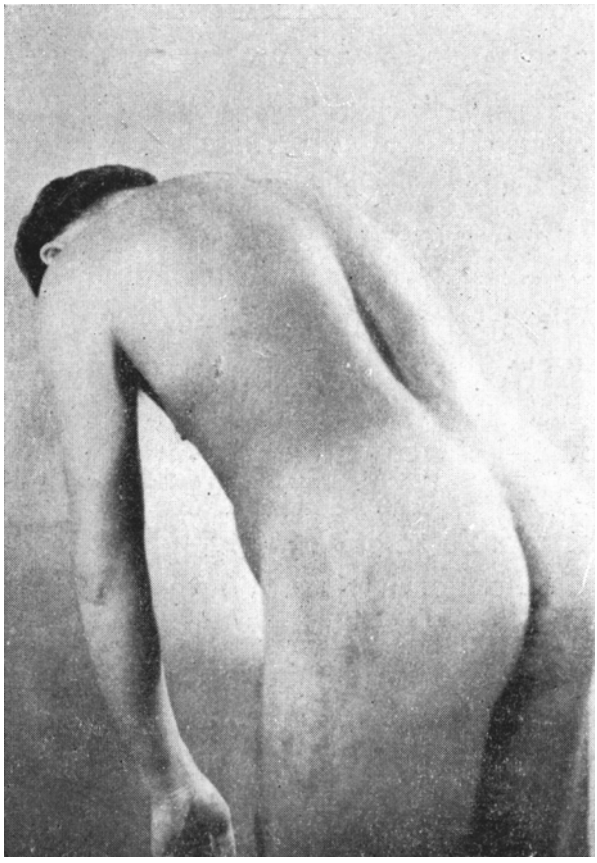

Fig. 11.4 Spine of the patient affected by AS. Sulcus dorsalis persistens is apparent in anteflexion

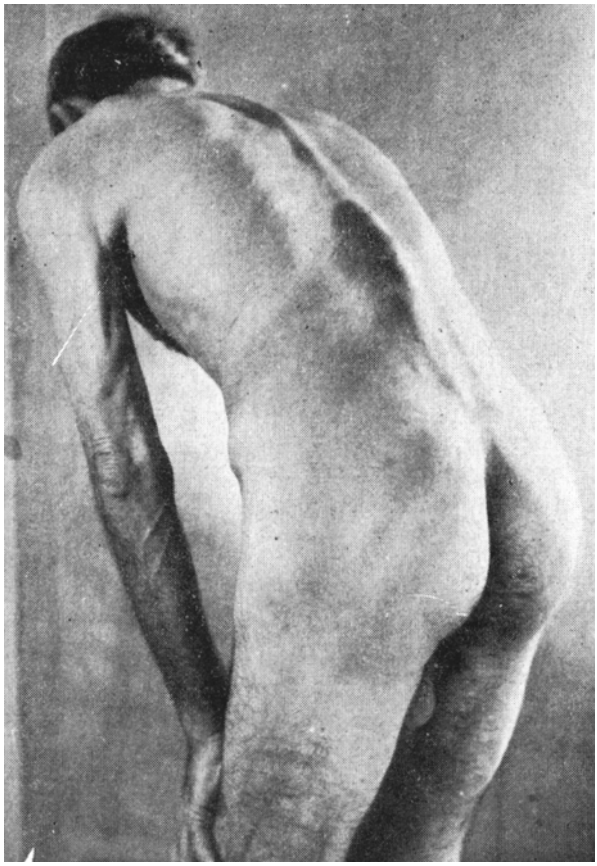

Fig. 11.5 Spine in ochronotic arthropathy is rigid and irregular, and contours do not change on anteflexion

Similarly, head movements are always limited in AS, often even in less advanced stages; in contrast we have only encountered with this phenomenon in two patients with ochronotic arthropathy who had severe articular changes, and in these cases, head mobility was not completely limited. General signs like higher ESR, weight loss, weakness or fever often accompany AS, but as it was mentioned before, they do not usually occur in ochronotic arthropathy.

Among X-ray signs, we would mention only affection of sacroiliac joints, where successive obliteration and synostosis occur in AS, while we do not see similar changes in these joints in case of ochronotic arthropathy.

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Analysis of X-Ray Symptomatology of Ochronotic Arthropathy in Spine

12

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Content

Skiagraphy still remains the principal method for the diagnostics of ochronotic arthropathy. Recognition of incipient secondary changes of joints due to deposition of ochronotic pigment improved with the advances in diagnostic technologies. Modern diagnostic imaging methods like CT and especially MRI enable identification of early specific signs of ochronosis, and thus they become important in the process of early determination of the diagnosis and in differential diagnostics of similar joint affections. Results of the studies comparing conclusions from classical X-ray and MRI examinations illustrate that both techniques are capable of detecting typical changes in cases when the diagnosis is known. In cases of newly diagnosed ochronotic arthropathy, it was confirmed that MRI is a more precise method in revealing and identifying lesions, not visualised in classical radiographs, like ligamentous lesions.

Intervertebral discs and paravertebral soft tissues are the first ones to be affected by deposition of ochronotic pigment. Ochronotic spondylarthropathy has characteristic radiological findings. Incipient X-ray signs already occur at the end of third, but more markedly at the beginning of the fourth age decade. The lumbar

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section is initially involved, and changes progressively spread to thoracic and later on to cervical section of the spine. The signs are initially limited to discrete disc lesions inducing change of signal intensity of affected discs in Tl- and T2-weighted images in MRI scans and narrowing of intervertebral spaces (lumbar-thoracic transition). Discrete reactive osteophyte formation at the margins of articular surfaces of vertebral bodies, slight calcifications in internal zone of annulus fibrosus in ventral part of intervertebral discs and insignificant calcification at the place of insertions of short vertebral ligaments, especially interspinal ones, can be present.

In more advanced stages of the pathological process, regressive disc changes increase, and calcifications become more intense. In advanced stages of ochronotic arthropathy, even synostoses of vertebral bodies develop with secondary straightening of lumbar lordosis. It is interesting that intensity and character of individual disc changes are not identical. Some of isolated discs are not affected, some other ones are desiccated, and some affected discs completely vanish.

In case of manifested ochronotic changes, significant osteoplastic appositions of the margins of articular surfaces of vertebral bodies arise, sometimes resulting in large bone bridging of an ankylosing hyperostosis type. Calcification of some of peripheral fasciculi of annulus fibrosus can have the features of pseudosyndesmophytes. Markedly sclerotised articular surfaces of vertebral bodies, irregular round translucencies with a thin marginal sclerotisation rim, so-called Bauer-Kienböck foci, can sometimes be visualised. These translucencies can also be demonstrated in ochronotic patients in superficial as well as deeper subchondral zones of large joints and also, e.g. in ischium.

A typical finding that can be best visualised in the lateral spine projection is the vacuum phenomenon. It creates linear or radial translucency in the disc area, often affecting multiple discs. This phenomenon is rarely visible in healthy individuals, somewhat higher frequency of the findings is in patients with osteoarthrosis, but it does not occur with such a high frequency and large extent as in patients with ochronotic spondylarthropathy. Early stages rapidly progress to advanced ochronotic discopathy when the skiagraphic changes in spine become specific and even pathognomonic. The spine can have the configuration resembling a bamboo rod (Resnick 1989).

Severe changes in intervertebral osteochondrosis type dominate. The density of calcified discs stands out in significant diffuse osteoporosis of adjacent vertebral bodies; however, thickening of bone structure resembling to pagetoid osteitis is not rare. Asymmetrical narrowing of intervertebral spaces leads to irregular small-arch scoliotic deformations of the spine. Not only discs, but also vertebral bodies are deformed after compressive fractures, and in some cases they are also

usurated. Massive hyperplastic ridges bridge the individual vertebrae. In cervical section, we encounter marked deformations of vertebral bodies. We observe local calcifications of longitudinal ligaments in lumbar region. Destructive changes of spine in advanced stages resemble to those in tabic arthropathy. In the differential diagnosis of disc calcification, findings of similar character on spine skiagrams, chondrocalcinosis and primary amyloidosis with massive deposition of amyloid and secondary calcification of intervertebral discs (Ballou et al. 1976) have to be taken into account.

In case of coincidence of ochronosis and AS, much less pronounced typical ochronotic changes were observed on X- rays than in other ochronotic patients with identically advanced stage of the disease. It is assumed that premature rigidity of spine caused by AS prevents development of ochronotic changes adequate to age in this area (Siťaj et al. 1956) (Figs. 12.1, 12.2 and 12.3).

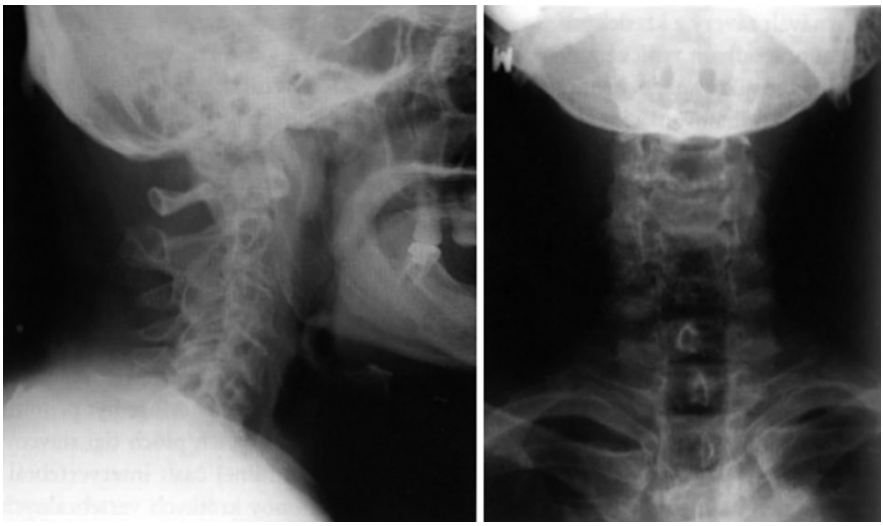

Fig. 12.1 Cervical spine: head in forward projection. Narrowing of intervertebral spaces with considerable subchondral sclerotisation of articular surfaces and non-homogeneous mild opacity of intervertebral spaces, uncovertebral arthrosis and arthrosis of intervertebral joints grade II in the whole part, more accentuated on the left side and in distal half of C-spine

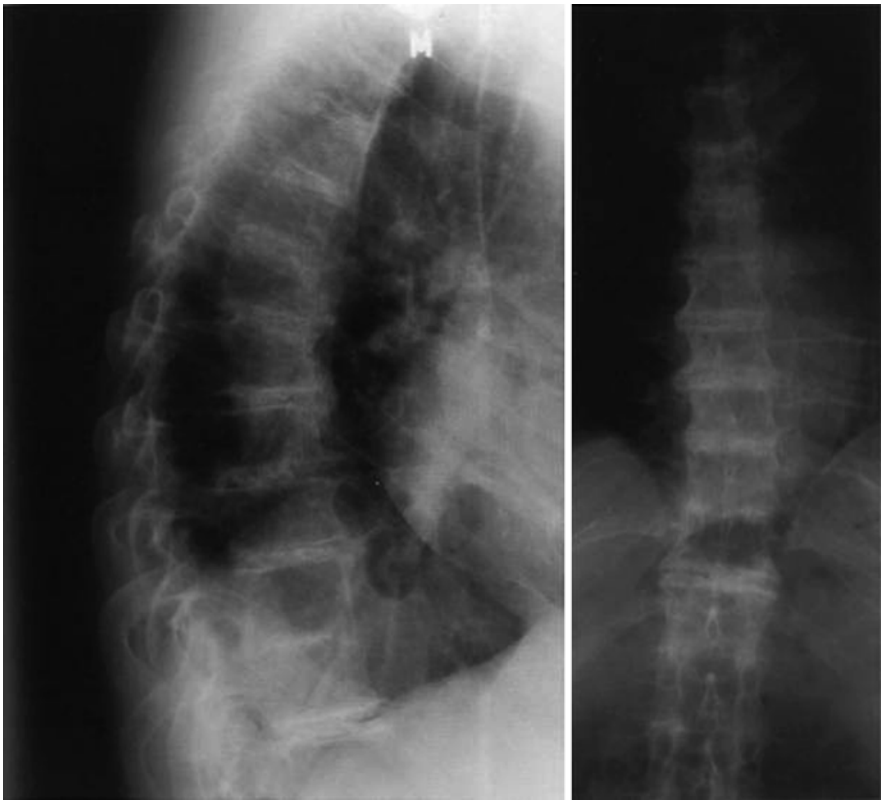

Fig. 12.2 Thoracic spine: considerable subchondral sclerotisation of articular surfaces of vertebral bodies deformed by frontal as well as thicker lateral beak-like osteophytes creating the picture of ankylosing hyperostosis; slightly reduced intervertebral space filled with central lamellar inhomogeneous calcifications in the whole part, even synostosis of vertebral bodies at Th 10–11 segment

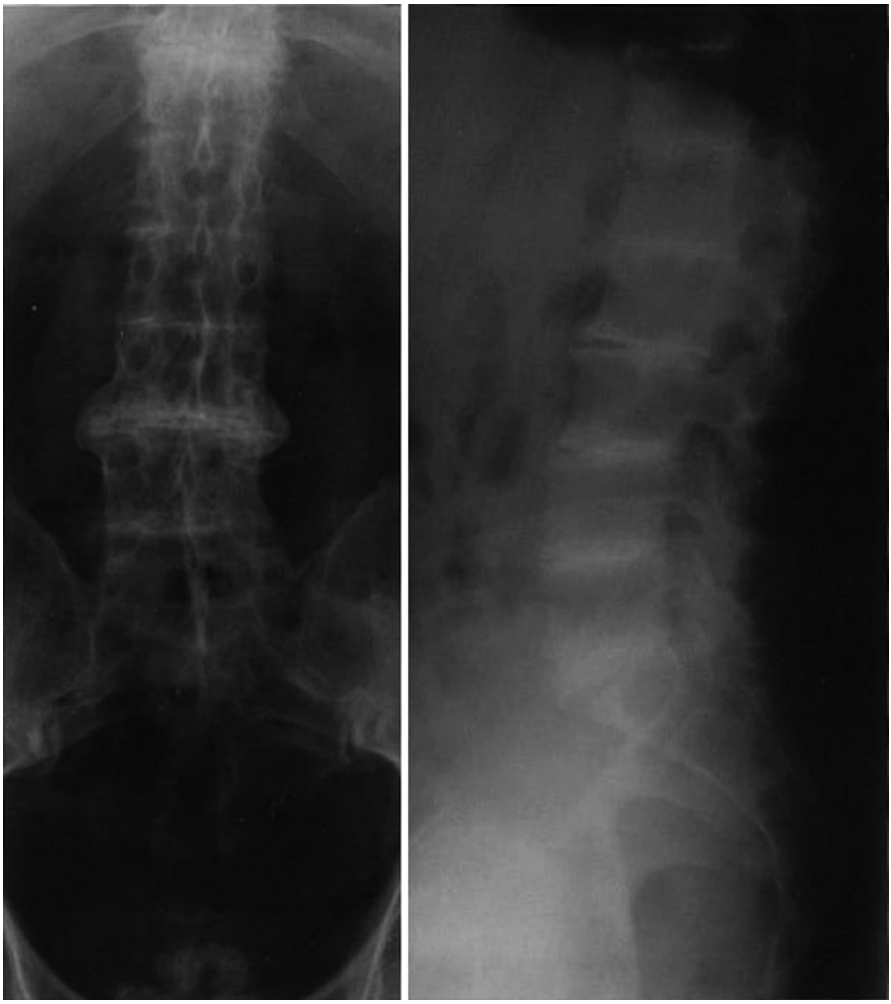

Fig. 12.3 Lumbar spine: straightened lumbar lordosis, polydiscopathy in the whole segment with considerably narrowed intervertebral spaces and vacuum phenomenon in L2–3 and L3–4 segments, rests of intervertebral spaces filled with inhomogeneous calcifications. Articular surfaces of vertebral bodies are sclerotic with marginal osteophytes. Marked apophyseal arthrosis, especially of distal part

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Clinical Manifestation of Ochronotic Arthropathy in Peripheral Joints

13

Tibor Urbánek, Štefan Kopecký, and Jozef Rovenský

Content

While the spine is affected in every patient suffering from ochronotic arthropathy and it dominates the arthrologic findings, involvement of some peripheral joints is frequent, but not inevitable (Lindušková et al. 1992; Hench 1948). It is characteristic that ochronotic arthropathy affects only large joints. From the point of occurrence frequency, the joints are affected as follows: knees (64 %), shoulders (42.3 %) and hips (34.6 %). It is typical that elbows as well as hand and feet joints are not affected by pathological process.

As in osteoarthrosis, the most affected peripheral joints in ochronotic arthropathy are the knees (Figs. 13.1, 13.2, 13.3 and 13.4). Both these diseases have similar symptoms in knee area. However, there are certain differences, especially onset of ochronotic arthropathy of knees in younger age (average age of 39 years), more rapid progression and more pronounced deformations. According to our experience, knee hydrops is much more frequent in ochronotic arthropathy (30.4 %).

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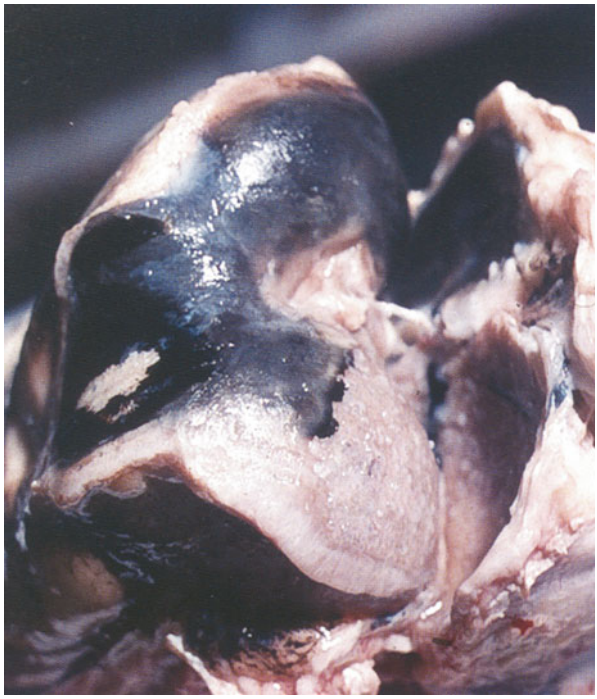

Fig. 13.1 Ochronosis. Open knee joint. Deposits of ochronotic pigment give the cartilage the blue-black colour

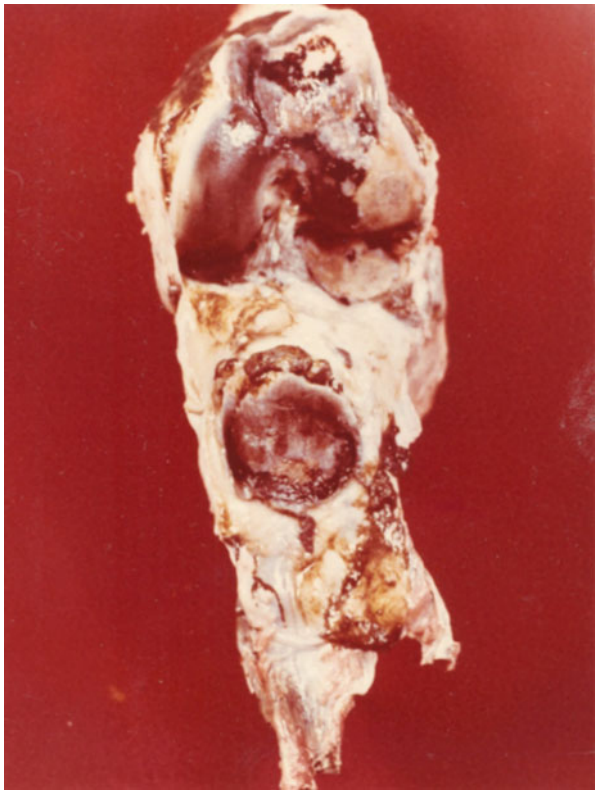

Fig. 13.2 Distal epiphyseal end of femur. Map-like erosions of articular cartilage that has black pigmentation on lateral condyle and facies patellaris. Dorsal area of patella has black pigmentation with smaller erosions of articular cartilage. Pedunculated pigmented corpuscles on apex and base

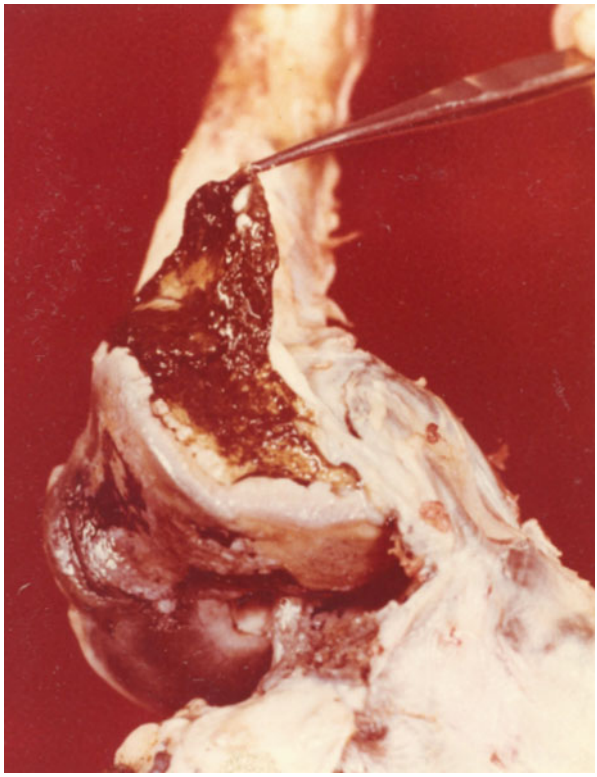

Fig. 13.3 Marked denudation of articular cartilage on lateral femoral condyle. Marginal osteophytes. Brown-black synovial membrane and cartilaginous corpuscles in it

There are the cases where hydrops is the first symptom of pathological affection of the knees. Besides the ochronosis, higher exposure of knee joints to mechanical factors definitely contributes to this symptom. In general, we can say that problems of the patients with ochronotic arthropathy concerning of knees do not differ basically from problems in osteoarthrosis. The symptoms appear slowly, pain is not initially strong, and it is induced by strain in this area. Some patients complain of numbness, inelasticity and infirmness that manifest during the first steps and relieves after joint activation. The disease progressively deteriorates, problems become more persistent and intense, and they are induced by even smaller mechanical stimuli. During the examination of affected joint, we can observe loss of the fine contours of knee, gradual thickening and development of an irregular shape. The volume also increases, and we can feel thickening of soft tissues on palpation, especially over patella which is more pronounced in the case of atrophic quadriceps muscle. During movement, we can observe crepitations on palpation as well as auscultation. Crepitations can rarely be very loud. In advanced stages of the disease, deformities escalate. The knee usually deviates to valgus position. Markedly prominent medial condyles form the characteristic look of this area. Mobility is more limited when compared to osteoarthrosis.

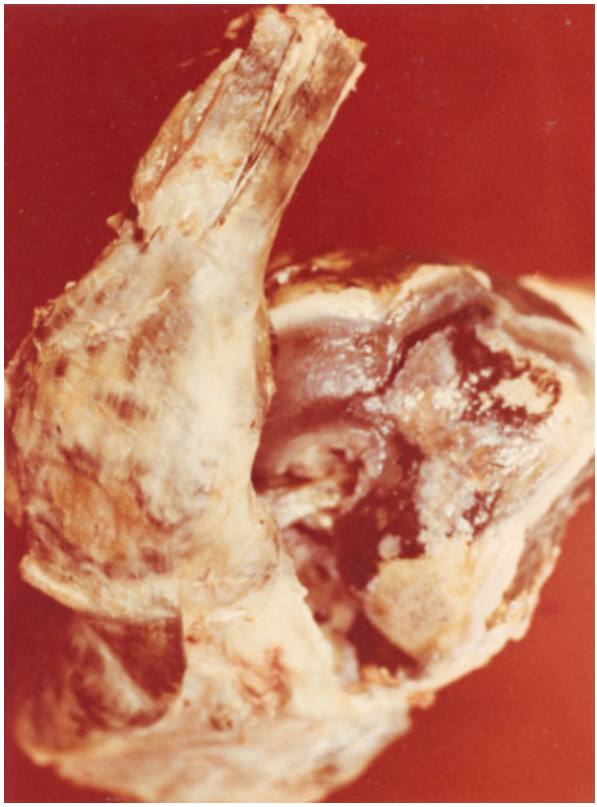

Fig. 13.4 Erosions of black-pigmented cartilage on patellar facet and on lateral femur condyle. Brown to black pigmentation of patellar ligament

Extension is more limited in osteoarthrosis, while mobility limitation in ochronotic arthropathy is caused by flexion reduction. Extension restriction is apparent in the further course of the disease. A complete clinical manifestation of ochronotic arthropathy in the knee area will be marked in 6–12 years from the disease onset. In contrast to the literature data (Zhao et al. 2009; Uebermuth 1928), we have never found an inflammatory character of knee involvement of a rheumatoid arthritis type or clinical signs of pannous synovitis in our patients. No patients complained of pain at rest. Volume expansion of the knee was not caused by inflammatory oedema, but by 'cold', slightly painful thickening of soft tissues. We also have not seen any knee deformities characteristic for rheumatoid arthritis. The soft tissues, namely, subcutaneous tissues around the joint, did not exert the symptoms suggesting panniculitis or cellulitis. These conditions are frequent accompanying symptoms of arthritic changes, especially in women. We do not usually detect venous insufficiency of lower extremities.

Shoulders are the second most commonly affected joints. Some patients only have vague muscle pain in the area of pectoral girdle. Nevertheless, in 42.3 % of cases, typical arthropathy of shoulder joints with mobility limitation developed,

approximately 10 years after the onset of the first symptoms of spine involvement. The pain initially occurs only during forced movements, and mobility becomes limited only successively and slowly, especially abduction and internal rotation, sometimes by 75 % of original motion range.

Involvement of shoulder joints is usually symmetrical, however not always of the same degree. Extremely affected shoulder joints were observed in a patient in whom long-term and repeated micro-injuries caused concentric symmetrical mobility limitation of shoulder joints by 80 %. X-rays revealed severe cartilaginous and bone changes of shoulder joints with massive hyperplasia of inferior margins of glenoid fossa. This was a clearly mechanically induced intensification of degenerative changes of arthrosis type. When compared to shoulder osteoarthrosis, these joints are affected much more frequently in ochronotic arthropathy. In large statistical sets of osteoarthrosis, shoulder joints are affected in 1–2 % of cases, while in our set of patients with ochronosis it was 42.3 %. The extent of joint affections with functional limitation is also much higher in ochronotic arthropathy. Concentric limitation of mobility can also be observed in some patients with AS, but it is induced by inflammatory process in soft tissues. Hip joints affected in advanced stages of ochronotic arthropathy are directly involved in disability. It is interesting that out of all our patients, nine of them had hip joints affected. Out of this number, five patients were females and four were males, suggesting higher prevalence in females. Occurrence of the first symptoms in hip area is hard to detect because the patients have only slight subjective problems and they cannot recall the time of onset. In contrast to other joint diseases, affection of hip joints in ochronotic arthropathy is rarely associated with mild pain; usually painless, slowly progressive mobility limitation dominates, similarly as in ankylosing hyperostosis. In more detailed analysis of mobility, we find reduction of abduction as the first symptom. Limitation of rotational movements, especially internal rotation, follows. In advanced stages, we can observe concentric limitation of movement excursions. When we compare affection of hip joints in osteoarthrosis, AS and ochronotic arthropathy, we can see a certain analogy in the onset and development of successive limitation of mobility – particularly limitation of abduction, rotation and extension (Siavashi et al. 2009; Forestier et al. 1951; Justensen and Andersen 1984) in all these diseases. The difference consists in nearly simultaneously limited flexion in ochronotic arthropathy, while this movement remains preserved for a long time in coxarthrosis and AS. Early onset of affection of hip joints and more rapid development of the disease with intense concentric mobility limitation that results in ankylosis and premature disability distinguish ochronotic arthropathy from genuine osteoarthrosis of hip joints. In three of our patients, severe flexion contracture developed in this area and caused directly their working disability.

Probably the biggest difference between these pathological conditions in hip joints is pain severity. In coxarthrosis, pain occurs as the first symptom in majority of cases. Initially it is periodic, and then it slowly becomes permanent and worsens with mechanical load and cold. In more advanced cases, the patient suffers more, not because of intensity but due to its persistency. As we mentioned before, pain is

not dominant in ochronotic arthropathy; it is only mild and often occurs only after mechanical stimuli and rarely at rest. Pain in ochronotic arthropathy differs even more from piercing intense pain that often occurs at rest in AS or other inflammatory affections of hip joints.

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Clinical and Ultrasound Evaluation of Enthesopathy in Alkaptonuria in Romani (Gypsy) Community

14

Isaac Jebaraj, Prasanna Jebaraj, and Shyam Kumar

Content

Patients from a Romani (Gypsy) community in the neighbourhood of Vellore in India, clinically diagnosed as spondyloarthropathy over a period of 8 years, were subjected to urine HPLC for homogentisic acid. Out of 40 patients, 12 (8 males/4 females) were positive for alkaptonuria. All the 12 Romanis presented with diverse symptoms of low backache, knee pain, ankle pain, etc. They came from the nearby Gypsy settlements from a radius of about 70 km, and all of them belonged to a homogeneous ethnic group of a population of 20,000. The results of the clinical and radiological investigations are summarised in Tables 14.1 and 14.2.

The ages of the patients ranged from 30 to 74 years with a mean age of 49 years. All patients had bilateral heel pain. Clinically, tenderness in the tendoachilles region was mild in 11 tendons, moderate in 3 and severe in 10 tendons. Tendoachilles rupture was clinically suspected in 7 out of the 24 tendons.

Ultrasound showed evidence of enthesopathy in all the examined tendons with findings ranging from early tendinosis to complete rupture. There was asymmetrical involvement of Achilles tendons in all the patients. The thickness ranged from 4.1

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Table 14.1 Clinical presentation and signs in the Achilles tendon

Patient no.	Age	Gender	Side	Heel pain	Thickening	Gap in the tendon
1	30	F	RT	+	+	–
			LT	+	+	–
2	37	F	RT	+	++	–
			LT	+	+	–
3	40	M	RT	+	++	+
			LT	+	++	+
4	46	M	RT	+	+++	–
			LT		+	–
5	40	M	RT	+	+	–
			LT	+	+	–
6	50	M	RT	+	+++	+
			LT	+	+++	+
7	61	M	RT	+	+	–
			LT	+	+	–
8	74	M	RT	+	+++	+
			LT	+	+++	+
9	50	F	RT	+	++	–
			LT	+	+	–
10	55	M	RT	+	+++	–
			LT	+	+++	–
11	60	F	RT	+	+	–
			LT	+	+	–
12	45	M	RT	+	+++	–
			LT	+	+++	+

F female, *M* male, *RT* right tendon, *LT* left tendon

to 16 mm with a mean of 7.2 mm. Loss of architecture was seen in 13 tendons (54 %). Calcification was present in 7 of 24 tendons (40 %). The retrocalcaneal bursa was enlarged in 14 heels (58.3 %), nodules were seen in 9 tendons (37.5 %) and calcaneal surface irregularity was seen in 9 feet (37.5 %) as well. Intrasubstance rupture (partial rupture) was detected in 6 tendons (25 %), and complete rupture was seen in 2 tendons (8.3 %). These tendon ruptures detected on ultrasound correlated with clinically suspected rupture with one extra tear being identified by means of sonography. However, clinically it was difficult to differentiate between partial and complete ruptures; four clinically suspected ruptures were actually partial tears.

All patients were given 1–4 g of ascorbic acid daily for a period of 2 months along with NSAIDS (Morava et al. 2003; Mayatepek et al. 1998). The non-rupture group and younger patients had excellent pain relief compared to the older age group with complete rupture of the Achilles tendon who experienced no significant pain relief. This could be attributed to the alteration of biomechanics in the ankle and subtalar joints in patients with chronic rupture. Surgical intervention was not attempted in the complete rupture group due to the lack of evidence of the beneficial role of surgery in AKU-related ruptures.

Table 14.2 Ultrasound observations in the Achilles tendon

Patient no.	Side	Thickness (mm)	Architecture	Calcification	Calcaneal surface	Nodules	Bursa	Tendon rupture	Grading
1	RT	5.0	Present	–	N	–	N	None	0
	LT	4.1	Present	–	N	–	N	None	0
2	RT	5.1	Present	–	N	–	E	None	0
	LT	4.1	Present	–	N	–	N	None	0
3	RT	10.0	Lost	+	IR	+	E	Partial	3
	LT	16.0	Lost	+	IR	+	E	Partial	3
4	RT	7.4	Lost	–	IR	–	E	None	2
	LT	4.7	Present	–	N	–	N	None	0
5	RT	4.3	Present	–	N	–	N	None	0
	LT	4.9	Present	–	N	–	E	None	0
6	RT	8.8	Lost	+	IR	+	E	Partial	3
	LT	9.2	Lost	+	IR	+	E	Complete	4
7	RT	4.5	Present	–	N	–	E	None	0
	LT	5.1	Lost	–	N	–	E	None	0
8	RT	10.0	Lost	+	IR	+	E	Partial	3
	LT	11.0	Lost	+	IR	+	E	Complete	4
9	RT	7.0	Lost	–	N	–	N	None	1
	LT	5.0	Present	–	N	–	N	None	0
10	RT	10.0	Lost	+	IR	+	E	Partial	3
	LT	8.0	Lost	+	IR	+	E	None	2
11	RT	8.0	Lost	–	IR	–	N	None	2
	LT	12.0	Lost	+	IR	–	N	None	2
12	RT	11.0	Present	–	N	+	N	None	2
	LT	37	Lost	–	N	–	E	Partial	3

RT right tendon, *LT* left tendon, *N* normal, *IR* irregular, *E* enlarged

Romanies are a migratory homogenous ethnic group whose origin is traced to the Indian subcontinent, over 100 years ago. The Romani language is of Indo-Aryan origin and has many dialects. There are about 12 million Romanies estimated globally (www.reocities.com/~patrin). In India, they are mostly concentrated in the three southern states, namely, Tamil Nadu, Andhra Pradesh and Karnataka. They do not seek medical help from the existing health centres since they resort to indigenous medicines. From our community health–oriented peripheral clinic, they were referred to the orthopaedic department due to back pain and polyarthralgia. These patients were further investigated for spondyloarthropathy. Several of them had soft tissue calcifications, palmoplantar pigmentation and evidence of kyphosis and peripheral arthritis. The plain radiograph of lumbosacral spine showed interdiscal calcification in ten of these patients. The diagnosis was confirmed by HPLC detection of homogentisic acid in urine. Since all of them had pain in the region of tendoachilles, they were subjected to ultrasound examination.

Enthesopathy is a disease process occurring at the site of insertion of muscle tendons, ligaments and aponeuroses into bones or joint capsules. Spontaneous rupture of the Achilles tendon has been associated with a multitude of disorders, such as inflammatory and autoimmune conditions, genetically determined collagen abnormalities, infectious diseases and neurological conditions. A disease process may predispose the tendon to spontaneous rupture from minor trauma. The pathologic findings of enthesopathy range from mild peritendinitis to full-thickness tendon rupture.

In ochronosis, there have been several reports of traumatic and spontaneous ruptures (Manoj Kumar and Rajasekaran 2003; Abe et al. 1960). Although it is the strongest tendon in the human body, the Achilles tendon is the most commonly injured tendon. The Achilles tendon transmits the force of the gastrocnemius and soleus muscles, which are the primary flexors of the ankle. A full-thickness tear is characterised by separation of the torn ends, a change of contour of the tendon, acoustic shadowing at the margins of the tear from sound beam refraction and adjacent hypoechoic tendinosis. A partial thickness tear will show some intact fibres, with the tendon often enlarged greater than 1 cm and containing abnormally hypoechoic or anechoic areas corresponding to the tear and associated adjacent tendinosis. Tendinosis is characterised by decreased echogenicity in a swollen tendon and loss of the regular linear pattern of tendon architecture with a more heterogeneous appearance and intrasubstance anechoic foci.

Plain radiography has limited role in diagnosing early tendinitis. MRI is the other imaging modality for the evaluation of tendoachilles enthesopathy. MRI and ultrasound have similar sensitivity in demonstrating superficial soft tissue and bony surface abnormalities. Ultrasound does not detect insertional bone oedema associated with enthesopathy (Balint et al. 2002).

Ultrasound as a modality has the advantages of availability, low cost and reliability, though it requires expertise. Ultrasound is unequivocally superior in the evaluation of enthesopathy compared to the clinical examination. More than 20 % of full-thickness tears can be missed clinically at initial presentation, and these can be diagnosed by ultrasound (Hartgerink et al. 2001). Fatty degeneration is visible

Table 14.3 'Vellore grading' of tendoachilles enthesopathy based on ultrasound findings

Grading	Ultrasound findings
0 – Normal	A normal Achilles tendon appears as a ribbon-like image; tendon fascicles (fibres) show a fibrillary pattern with alternate hypoechoic and hyperechoic lines, thickness less than 5.2 mm
1 – Early enthesopathy	Loss of fibrillary pattern of the tendon (haziness) (Fig. 14.1)
2 – Late enthesopathy without tear	Loss of fibrillary pattern with nodules: increased thickness, calcification, intact fibres (Fig. 14.2)
3 – Partial tear	Partial disruption of the tendon fibres with or without a mixture of fluid, fat and/or granulation tissue (Fig. 14.3)
4 – Complete tear	Total disruption of the tendon fibres with or without a mixture of fluid, fat and/or granulation tissue (Fig. 14.4)

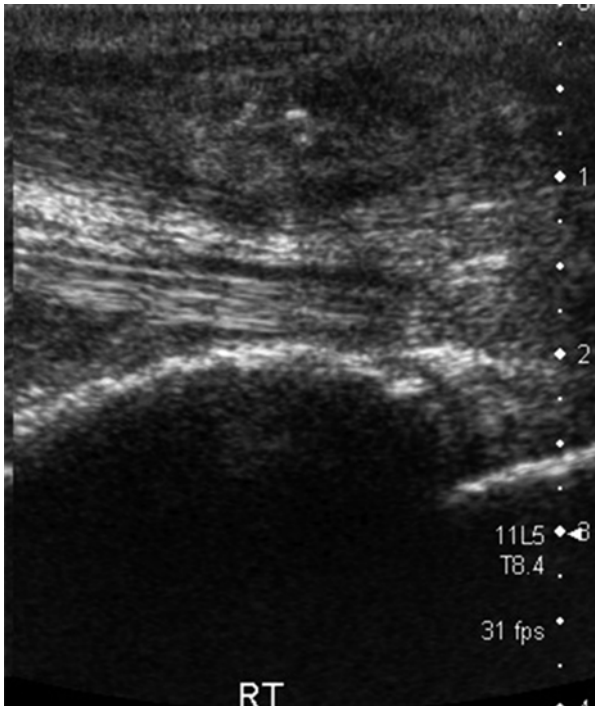

Fig. 14.1 Grade 1 – Early enthesopathy. Loss of fibrillary pattern of the tendon (haziness)

late on MRI, while it can be detected earlier by ultrasound. The ultrasound has the advantage over MRI in detecting early calcification at the tendinous insertion.

This study in alkaptonuria patients showed the similar ultrasound features of enthesitis that were described in the other articles (Hartgerink et al. 2001; Jamadar et al. 2002; Kamel et al. 2003, 2004). The distribution of ultrasound findings was asymmetrical in both tendons of the patients. We would like to introduce a grading system based on ultrasound findings (Table 14.3). This will help by assessing the status of the tendon at presentation and by comparison on follow-up.

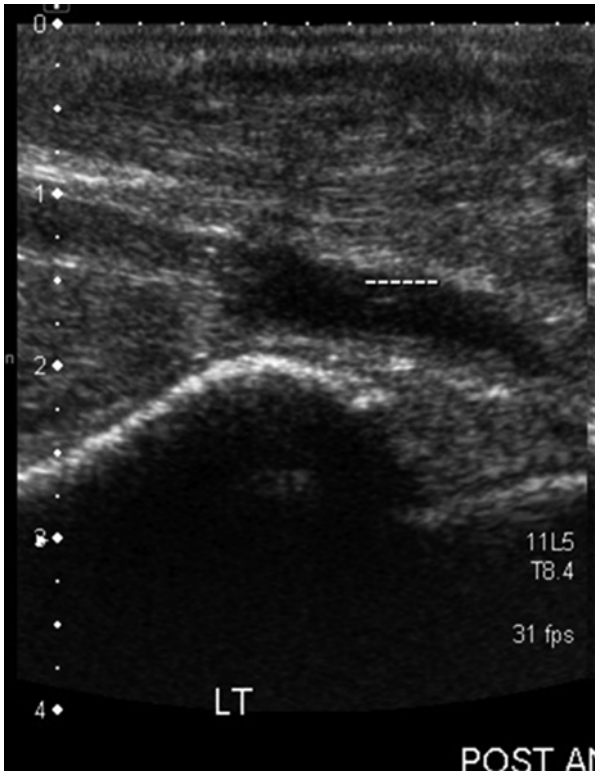

Fig. 14.2 Grade 2 – Late enthesopathy. Increased thickness, calcification, intact fibres

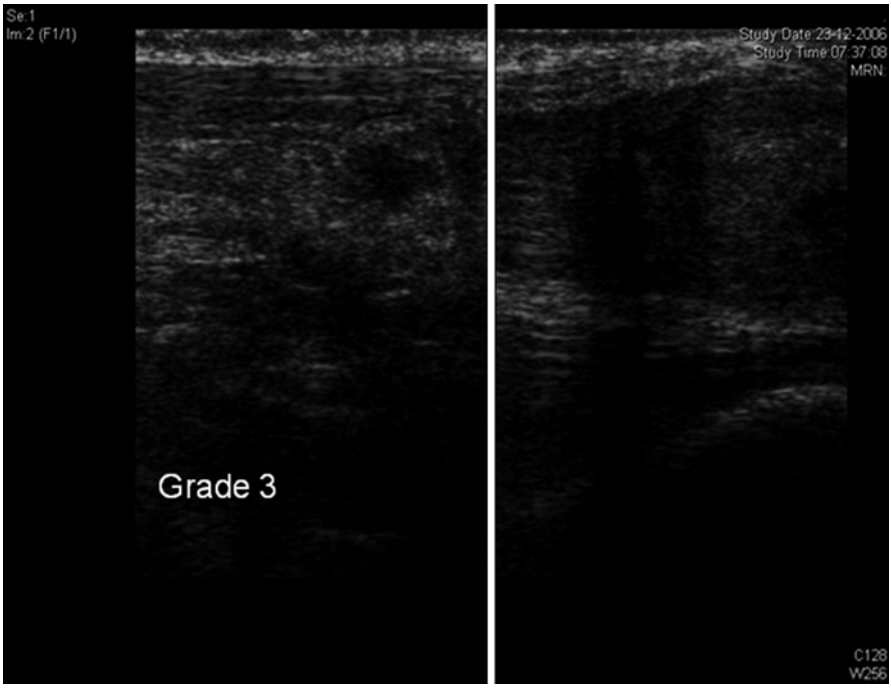

Fig. 14.3 Enthesopathy grade 3. Partial tear

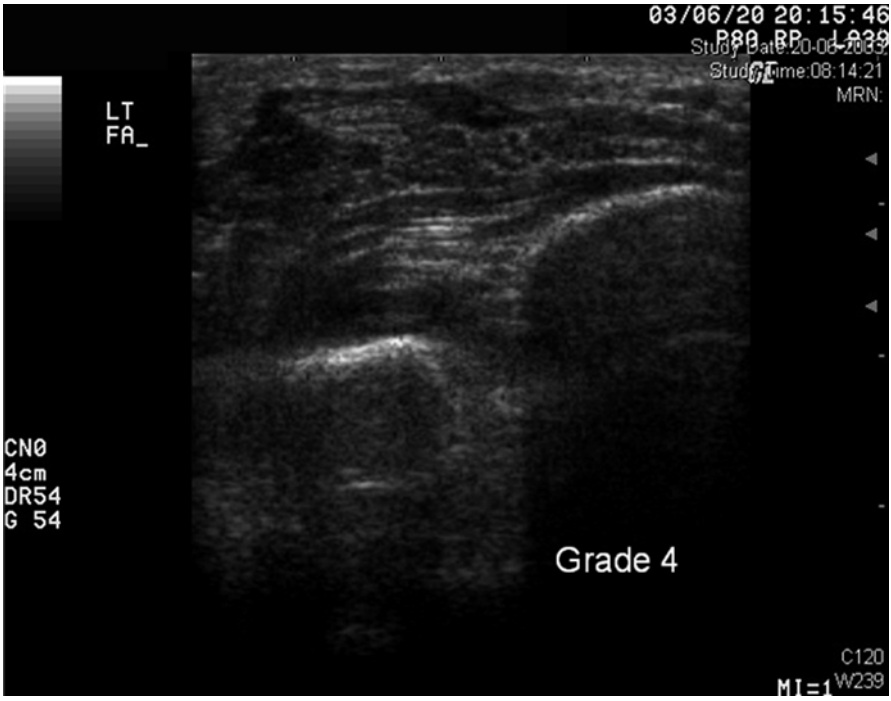

Fig. 14.4 Enthesopathy grade 4. Complete tear – total disruption of the tendon fibres

Summary

- A higher occurrence of alkaptonuria is found in the Romani ethnic group in India. Conservative management gives satisfactory result in chronic rupture of the Achilles tendon in alkaptonuria.
- Degenerative changes in the tendoachilles are common in these patients due to ochronotic pigment deposition. The ultrasound is a very useful modality in the evaluation of early stages of enthesopathy.
- Ultrasound findings are similar to those reported in the enthesopathy due to other causes, but changes are more pronounced in alkaptonuric patients (Kamel et al. 2003).
- The 'Vellore grading' is introduced to quantify the severity of enthesopathy, may be a useful tool in ultrasound evaluation and may be used in all tendoachilles enthesopathies.

An early detection of the enthesopathy will help in appropriate management of the disease and will be useful in reducing the disease-related morbidity secondary to enthesopathy.

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Analysis of X-Ray Symptomatology of Ochronotic Arthropathy in the Area of Peripheral Joints

15

Jozef Rovenský, Mária Krátka, and Tibor Urbánek

Radiological findings on peripheral joints in ochronotic arthropathy are identical with the findings in osteoarthrosis. However, arthrotic involvement of peripheral joints in patients with ochronosis, when compared to non-ochronotic arthropathy, occurs with high frequency at age when the manifestation of joint lesions is usually not assumed, and it often has much more progressive course. Areas not typical for degenerative affection without the history of injury are often involved – glenohumeral joint, sacroiliac joints and symphysis. In ochronotic arthropathy, articular slits become narrower in an untypical way – symmetrically in hip and shoulder joints and asymmetrically in knees, with isolated involution of the lateral compartment. Collapse and fragmentation of bones with the formation of free intra-articular corpuscles occur quite frequently. The most frequently affected peripheral joint is the knee. X-rays show the early manifested arthrotic changes. Articular slits narrow either symmetrically or asymmetrically with isolated involvement of lateral compartment of tibiofemoral articulation. Osteophyte deformations are similarly present on the margins of sclerotised articulator surfaces of lateral condyles of the femur and tibia. The typical findings are also enthesopathic ossifications on patellae and tuberositas tibiae, occasionally calcifications in menisci, metastatic ossifications of synovial chondromatosis, collapse and fragmentation of bones, free intra-articular corpuscles and subchondrally localised demarcated translucencies, most frequently in the area of intercondylar eminence – Bauer-Kienböck foci (Fig. 15.1).

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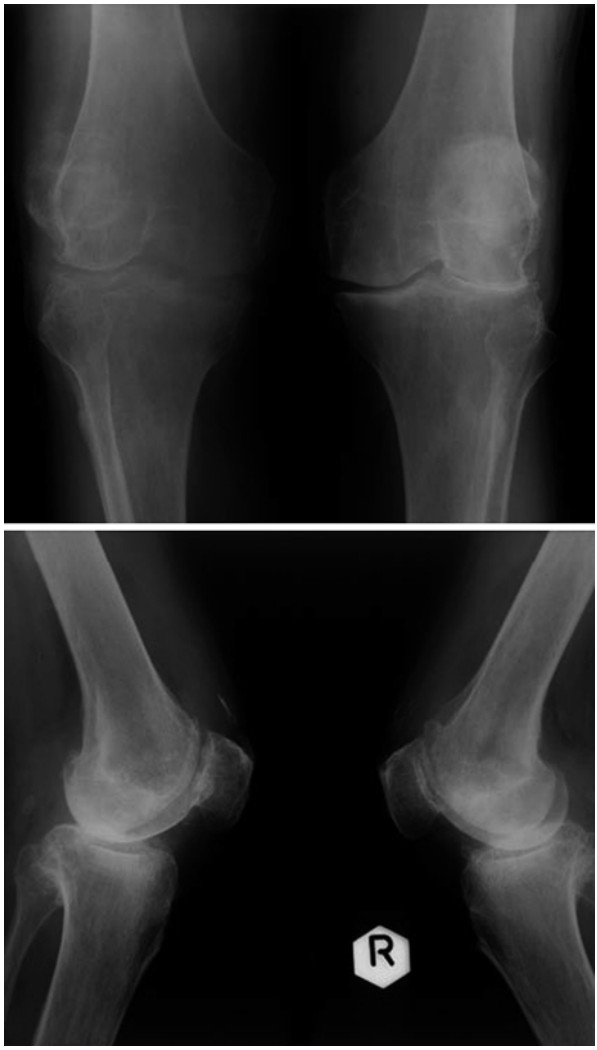

Fig. 15.1 Knees: varus position in both knees, subchondral sclerotisation and asymmetrical narrowing of tibiofemoral articular slit. Changes are accentuated in the lateral part of articular slit of the left knee with its narrowing to the minimum. Mild impaction and fragmentation of medial condyle of the right tibia. Osteophytes of articular surface margins, accentuated in patellofemoral area on the right side. Hyperostose rim on patella anterior surface

In shoulder joint, ochronosis is manifested most frequently as typical osteoarthrosis sometimes with atypical secondary finding. Numerous fibro-ostoses on the tuberculum majus or acromioclavicular and ochronotic foci localised in deeper structure of the head and neck of humerus and neck of scapula in supraglenoid area can be the atypical findings. Insignificant manifestations of osteoarthrosis are represented by spike-forming of the inferior margin of glenoid fossa and a small osteophyte on the lower pole of humeral head. This finding rapidly progresses to advanced stage characterised by considerable narrowing of glenohumeral articular slit, significant osteoplastic changes as well as possible mushroom-like flattening and fragmentation of humerus head (Fig. 15.2).

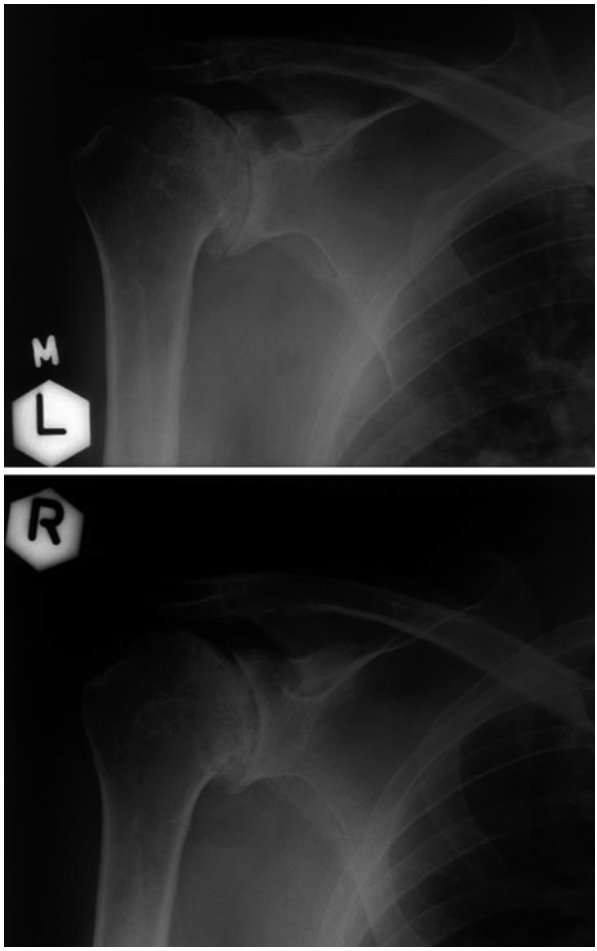

Fig. 15.2 Shoulders: Position of heads of both humeri in subacromial retraction, with sclerotisation and enthesophytes in the area of major tubercle – periarthropathia humeroscapularis (PHS) bilaterally. Narrowing of articular slit in humeroscapular joint to the minimum with slight incongruence and subchondral sclerotisation of articular surfaces, with osteoplastic rim of their margins that creates exostotic formation at the caudal margin of glenoid fossa. The humeral head is mildly flattened on the right side, with irregular contours, shallow usurations and cystoid translucencies in deeper structure

In the hip joint, ochronosis manifests as prematurely occurring osteoarthrosis that exerts not only rapid progression, but it has also the signs of avascular osteonecrosis of the head of femur. In severe cases of advanced ochronotic arthropathy, the epiphyses of femurs are mushroom-like, flattened with wedging of the neck into bionecrotic head (Fig. 15.3). Accentuated longer and thicker fibro-ostoses on the greater trochanters of femurs, on ischial tuberosities, symphyses and partially on the margins of ilium alae are quite frequent. Free intra-articular corpuscles and Bauer-Kienböck bone foci in supraacetabular area and on site of tendinous insertions can rarely be present. Sacroiliac slits show symmetrical bilateral sclerotisation of subchondral bone of adjacent articular surfaces (Fig. 15.4).

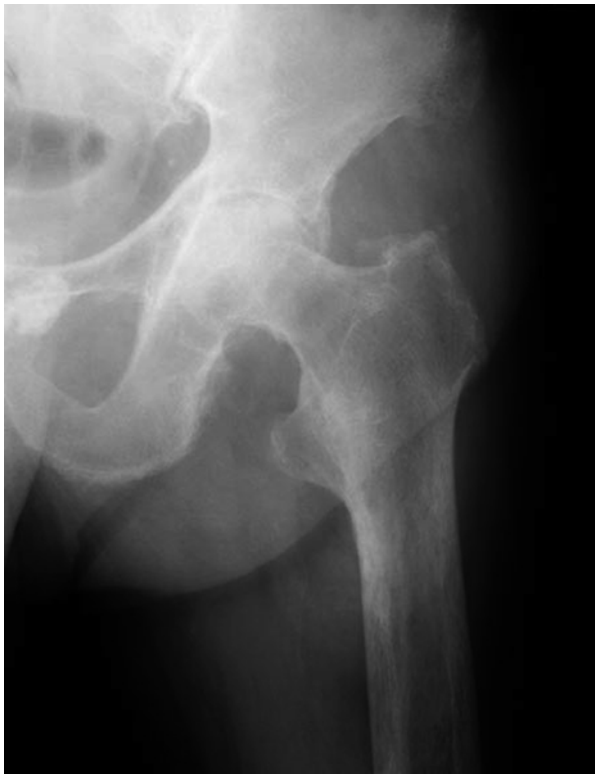

Fig. 15.3 The right hip joint: articular slit of hip joint is narrowed nearly to the minimum in mild craniolateral migration and protrusion of the acetabulum. Hyperplastic osteophyte rim of the margins of markedly sclerotic articular surfaces, enthesopathic ossifications of both trochanters, ischium tuberosity and ilium ala

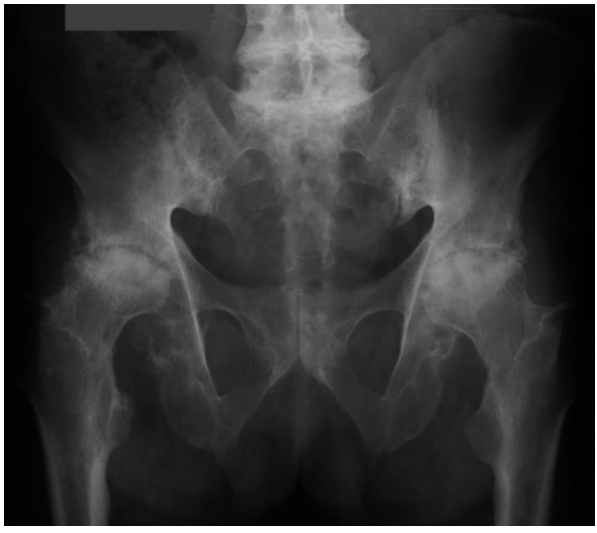

Fig. 15.4 Pelvis: SI joints are irregularly narrowed, contoured by non-homogeneous thicker margin of sclerotisation. Heads of both femurs are flattened; articular spaces are narrowed to the minimum due to craniolateral migration. Articular surfaces of the femur heads have irregular surface with unevenly located defects of bone contour with numerous geodes in deeper structure. Osteosclerotic thickening of acetabular tegmen margin bilaterally

Ochronosis in symphysis area results in wearing of articular surfaces. Contour defects, slit narrowing, subchondral sclerotisation, marginal osteophytes, extensive fibro-ostoses or ossifications of obturator membrane are visualised.

The small joints of the hand and feet are usually not affected. In rare cases when ochronosis is manifested clinically as well as on X-rays of hand and feet joints, the findings look like osteoarthrosis.

The radiological picture of ossifications of tendinous insertions that often occur in ochronotic patients does not differ basically from the picture of common degenerative fibro-ostoses. However, ochronotic fibro-ostoses are slightly more accentuated and thicker and occur more frequently than in patients who do not suffer from ochronosis. Tendinitis and spontaneous ruptures of tendons are relatively frequent findings in these patients.

Classical skiagraphic methods have limited usefulness in the diagnostics of early tendinitis, while ultrasonography and *MRI* are capable of detecting early calcifications and increased tendon thickness. *MRI* has an additional advantage of displaying the insertional bone oedema associated with enthesopathy. Nevertheless, according to the results of recent studies, ultrasonography is available, cheap, reliable and equivalent or even more sensitive compared to *MRI* in the diagnosis of early enthesopathy, calcifications and ruptures of tendons.

Manifestations of Alkaptonuric Ochronosis in the Eye

16

Tibor Urbánek and Jozef Rovenský

Content

The eye has many bradytrophic tissues that do not contain blood vessels and have slow metabolism. Similar to other metabolically inert tissues in the body, they are apparently susceptible to pigment deposition. Pigmentation in ochronosis is most apparent on the scleras and cornea, less often in the bulbar conjunctiva, tarsus and eyelid skin. We still cannot explain why ochronotic pigment is not deposited in bradytrophic tissues of the internal eye, parts like corpus vitreum and lens as well. It should be noted that pigment is not deposited in these parts of the eye in other metabolic and endocrine diseases as well.

Sallmann (1926) was the first ophthalmologist who drew the attention to the problem of eye ochronosis. Later on, this problem was discussed in rare case reports based on accidental findings. A systematic review was not available, and standard ophthalmologic textbooks (Lichtwitz 1932) deal with this issue only briefly.

According to literature review, ochronotic pigmentation of the eyes is found in about 2/3 of ochronosis cases. In the group of our patients, eye ochronosis was found in 70.5 % of the cases. On the basis of rare observed cases, it is generally stated that eye ochronosis begins at the average age of 30 years (Sallmann 1926).

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Identification of the first eye pigmentations in medical history is very difficult because discrete initial changes can be seen only during a specialised examination. We assume that ochronotic eye pigmentation can occur in young or even paediatric ages. We followed an 8-year-old boy with alkaptonuria who had an apparent pigment spot on the sclera of one eye. Eye ochronosis can also be beneficial in detecting alkaptonuria in black people (Salvi 1950).

As in other organs of the human body, ochronotic pigment forms by imbibition of the tissues with homogentisic acid and its polymers (Salvi 1950; Smith 1942). It is interesting but also important from the point of pigment formation that eye ochronosis is localised in those parts of the sclera, cornea and conjunctiva that are in the lid slit. The explanation of this phenomenon is difficult, but light and microtraumatic factors might play the role.

From the point of overall conception of the development of organ ochronosis, the significant observation is that ochronotic pigment is deposited outside the lid slit only at those places where scleras are perforated by ciliary blood vessels (emissary). We have carried out detailed analysis of eye findings in 11 patients with more or less pronounced ochronotic eye pigmentation.

The changes are most marked on the scleras where dark brown pigment can be best seen on white background (Fig. 16.1a, b). That is the reason why previous scientific papers dealing with the issues of alkaptonuria and ochronosis describe nearly exclusively only the changes on scleras.

According to our findings, the first traces of pigment appear in a horizontal plane 4–6 mm distance from the cornea margin, i.e. approximately at the places where the tendons of the oculomotor muscles are inserted to the scleras. They have a pale grey colour, and they progressively change to dark brown or a violet colour during a slow growth. Pigment colour is dependent on the depth of its deposition in sclera layers. The more superficial the pigmented particles are, the more marked their brown-violet colour is. Pigment spots that are located deeper have grey to blue shade. The colour of pigmented spot is the richest in its centre and paler in marginal parts, suggesting pigment deposition by means of apposition. The shape of the spots is diverse. Round, oval and more or less irregular shapes can mostly be seen.

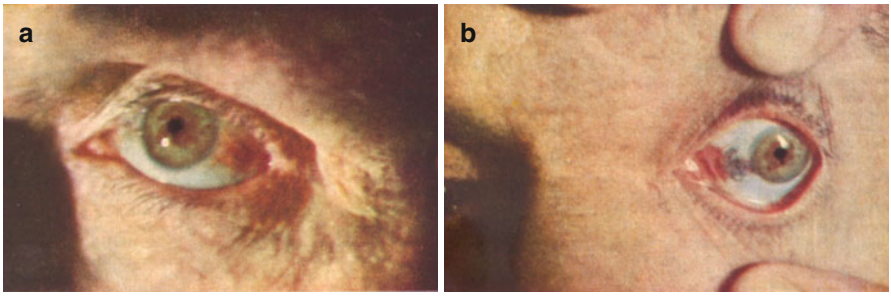

Fig. 16.1 (**a**, **b**) Ochronotic pigmentations on scleras in patients affected by ochronotic arthropathy

In advanced stages of ochronosis, the whole sclera has a dark grey shade. This phenomenon was sporadically described even without demarcated pigment spots (Sallmann 1926).

In addition to sclera, ochronotic pigment also appears in the conjunctiva and cornea which can often be overlooked during common non-ophthalmologic examination. In conjunctiva, small granules of brown pigment are deposited in the subepithelial area either in an isolated manner or in small groups that are irregularly spread or sometimes forming chain-like figures. In case of more abundant occurrence of pigment granules, they are positioned in circles or semi-circles (Sallmann 1926; Smith 1942). In the cornea, we can observe dark brown granules along temporal and nasal margin in various depths, reaching 1–3 mm to the cornea centre. In contrast to some authors (Smith 1942), we consider ochronotic pigmentation of the cornea as a consistent, not just secondary sign in advanced stages of ochronosis. In this context, we point out that similar pigmentation of the cornea does not occur in any other disease, and therefore, it is pathognomonic for ochronosis (Fig. 16.2).

While examining patients with eye ochronosis, we have gained the impression that the pigment continuously passes from conjunctiva to cornea and is deposited close to the pericorneal network. According to Bürger and Schulze (1953), the mechanisms of development of ochronotic pigmentation of the cornea and gerontoxon in the elderly are similar. In tarsus and eyelid skin, ochronotic pigment is deposited more seldom (Šrámek 1937). In everted eyelids, the pigmented tarsus can be seen through the conjunctiva as a brown shade.

As we mentioned before, 70.5 % of our examined patients had ochronotic changes of their eyes. The high number of patients investigated allowed us to observe various stages of ochronotic changes. It is clear that these changes have a progressive character over time.

In this group of patients, we present an 8-year-old boy in whom we observed apparent ochronotic pigmentation on the sclera. We have not found a similar case in the available literature. In three of our patients, we observed conjunctival and corneal deposits without colouration of scleras. In other seven patients over the age of 50, eye ochronosis was completely developed within the localisation on the scleras, conjunctivae and cornea. We have not found changes on the tarsus in any case. When we compare the extent of ochronotic changes of the eyes with the stage of arthropathy, we do not see any parallels, and relatively severe joint affections are often associated with only minimal eye changes.

Diagnosis of ochronotic pigmentation in the eyes is not difficult, but it is also important to consider exogenous ochronosis in chronic poisoning with phenol substances. We have to exclude all conditions resulting in eye pigmentations, namely, chronic arsenic and lead poisoning, longer use of adrenaline in the form of eye drops, conditions after scleritis, congenital melanosis of scleras, generalised argyrosis, Addison's disease and blue scleras in osteopsathyrosis. Characteristic urinary finding described in the particular chapter is decisive for the diagnosis.

Malignant degeneration of tissue imbibed with ochronotic pigment has not been observed. Skinsness (1948) enucleated the only eye by mistake (the patient lost the other eye due to injury) due to suspicion of melanosarcoma. After the patient's

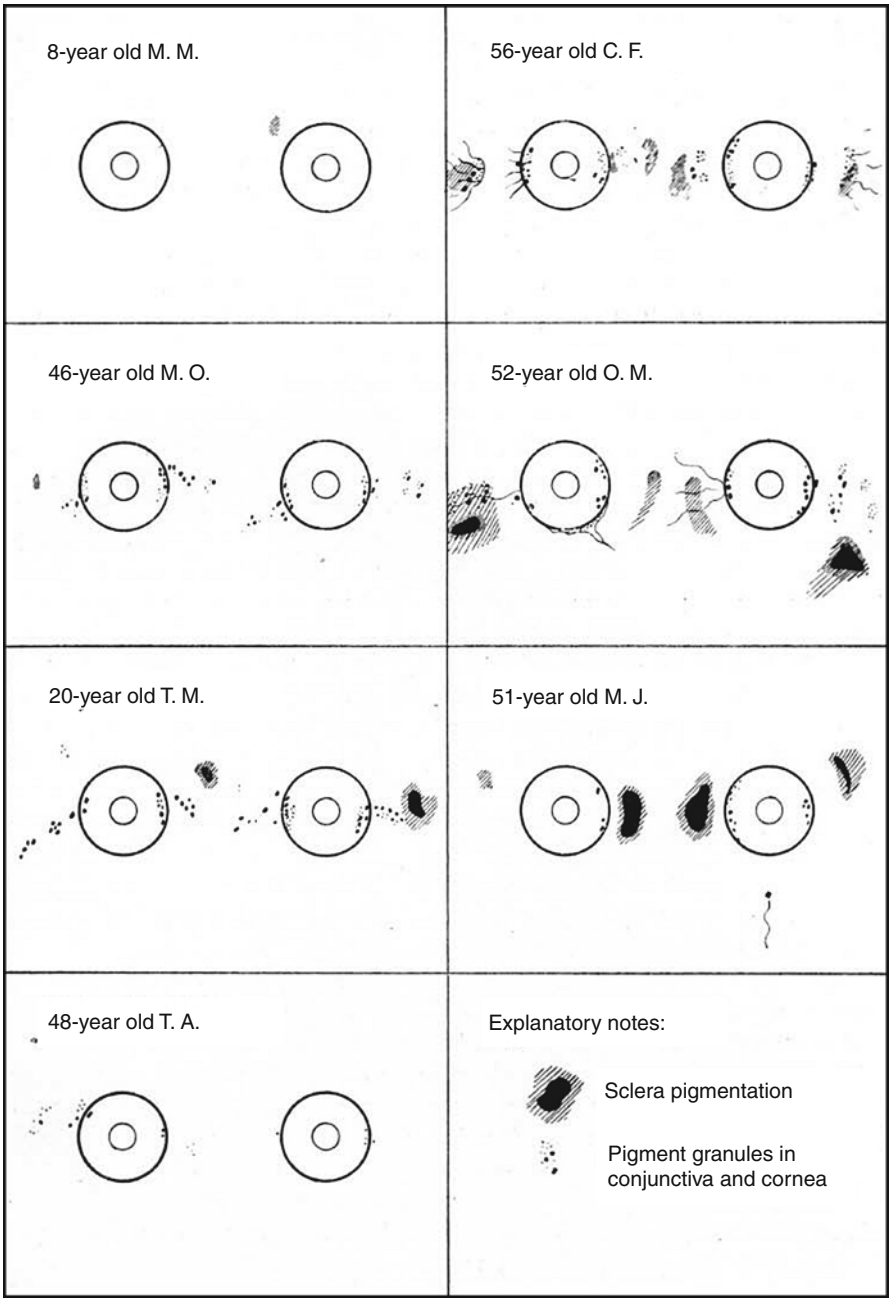

Fig. 16.2 Schematic findings of ochronotic changes in the eye

accidental death, it was revealed that it was alkaptonuric ochronosis with eye pigmentations. According to our experience, alkaptonuria does not cause vision disturbances, irritation or inflammatory eye changes. The patients are asymptomatic and do not visit physicians due to eye symptoms. Salvi (1950) found chronic glaucoma in two ochronotic patients who were siblings. He suggested that it was an accidental coincidence of alkaptonuria with a familial form of glaucoma.

In some patients, alkaptonuria was diagnosed on the basis of accidental ophthalmologic examination (Smith 1942). In our ophthalmologic clinic, we have also diagnosed alkaptonuric ochronosis in a patient who came due to presbyopia. It is important to think of this disease if pigmentation of the anterior segment of the eye is found because this disease is not as rare as it is generally quoted in the literature. Therefore, it is necessary to exclude alkaptonuria by the appropriate urinalysis if pigment anomalies in the eyes are found.

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Symptoms of Alkaptonuria and Ochronosis in the Ear

17

Tibor Urbánek and Jozef Rovenský

Content

Little attention has been paid to alkaptonuria and ochronosis in otorhinolaryngologic literature until now in spite of the fact that auricle changes are typical and pathognomonic and belong to the first symptoms of the disease and they often lead to the diagnosis of this rare metabolic disorder.

Characteristic colouration and consistency change of auricle cartilages are described in nearly all the literature on this disease.

Uebermuth (1928) and other authors histologically examined ochronotic auricles. The concha, anthelix and helix had ochronotic colouration. The pigment was located in the subperichondral zone on the concave area of the auricle, mostly in the intercellular substance and also inside cells at the places of more intense colouration. The auricle was encrusted with calcium, and it was fragile and brittle, which is rare in hyaline cartilage. Pomeranz et al. (1941) described deposition of calcium in the helix, fossa triangularis and anti-tragus on X-ray of the ochronotic auricle. Brunner (1929) carried out a very detailed histological examination of the temporal bone of a patient presented by Bauer and Kienböck (1929). Clinical examination revealed retracted tympanic membranes. The sound-perceptive apparatus did not

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exert any changes. Deposition of pigment in bone and membranous parts of the temporal bone was found in the biopsy. It could be seen on the tympanic membrane, manubrium, promontorium (Fig. 17.1a, b), the internal auditory meatus which was widened, cochlea periosteum and endosteum, in the utriculus and canals. The dura was also coloured to a dark blue shade. Rich pigmented fibrous bands were found in the antrum. Otosclerotic foci that did not contain pigment were found in the patient. On the basis of the analysis of pathogenesis and clinical symptoms of ochronosis, it can be assumed that the harmful effect of homogentisic acid is not limited only to the auricular cartilage but to other tissues of the ear where its derivatives are deposited. Disintegration of the tissues and deposition of derivatives in the injured parts as well as the toxic effect of homogentisic acid as a phenol substance would impact on clinical manifestation of ochronosis in ear. Hearing defects are also well known in other metabolic disorders (diabetes, gout, myxoedema, Hand-Christian-Schüller disease, etc.) as well as in toxic injury of sound-perceptive apparatus with phenolic substances (phenol, salicylic acid).

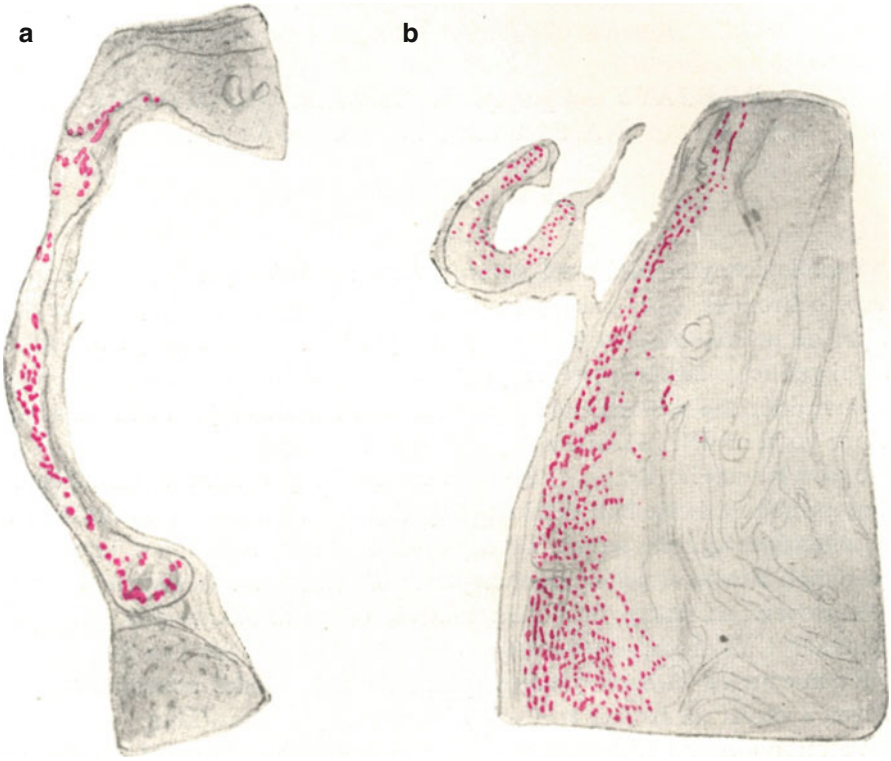

Fig. 17.1 (**a**) Section of the oval window: unstained histological section, pigmented bone cells on stapes base. (**b**) Promontorium section: unstained histological section, pigmented cells in periostal layer and on stapes head

In clinical examinations of 17 patients with manifested ochronosis, there have been the following findings in the organ of hearing. Auricular changes are visible, they are typical, and it can be stated that they are one of the most constant symptoms of ochronosis in the course of alkaptonuria. They often lead to the diagnosis of the primary disease. Pigmented cartilage of the auricle appears darker on transillumination. Salts are deposited in damaged tissue at affected places, forming rugged blue to violet figures showing through the skin. These changes appear in adult age, usually before the onset of the first joint symptoms. They arise unnoticed so that the patients themselves are frequently not aware of them until other people often warn them of the blue shade. Painless, hard and rough tubers with a wide base looking like infiltrates can be palpated in cartilage. They are firmly bound to the base and show through a fine skin as brown/dark blue/violet figures. The first rugged prominences can be observed on the lower crus of the anthelix, later on the whole anthelix, in the triangular fossa, less frequently on the helix crus and in the cavum conchae, cymba and tragus. In more advanced stages, the whole anthelix and later on the whole auricle are harder on palpation and less elastic and lose their configuration over time. Auricle deformities rarely occur (Fig. 17.2).

In our patients, we found blue to violet prominences only on the concave area of the auricle. In this area the perichondrium is bound to cartilage more firmly than on the convex side. Susceptibility to ochronotic changes may be associated with microinjuries during the cleaning and washing of ears and due to their exposed location. While washing the auricles, fingers frequently touch just the above areas with a predilection for ochronosis. Patients themselves frequently report that some minor injuries like blows, frostbites, etc., preceded pigmentation. Symmetrical localization on both sides is relatively frequent.

Opacities with sharp contours on auricle X-rays resemble calcifications (Fig. 17.3a–c). This finding was present only in the advanced stages of ochronosis when the cartilage was already less elastic and harder on palpation. X-rays were normal in initial stages of the disease as well as in the presence of apparent bluish colouration of the auricles.

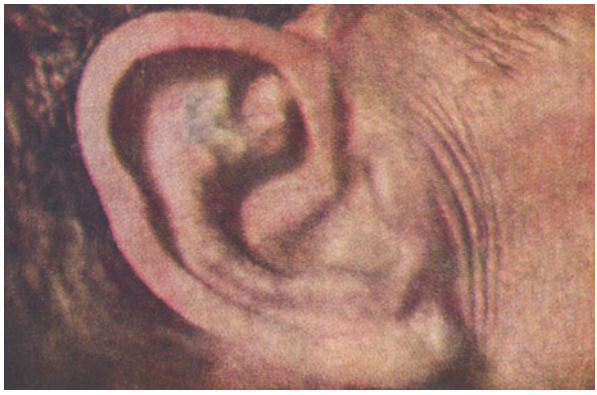

Fig. 17.2 Ochronotic changes on the ear

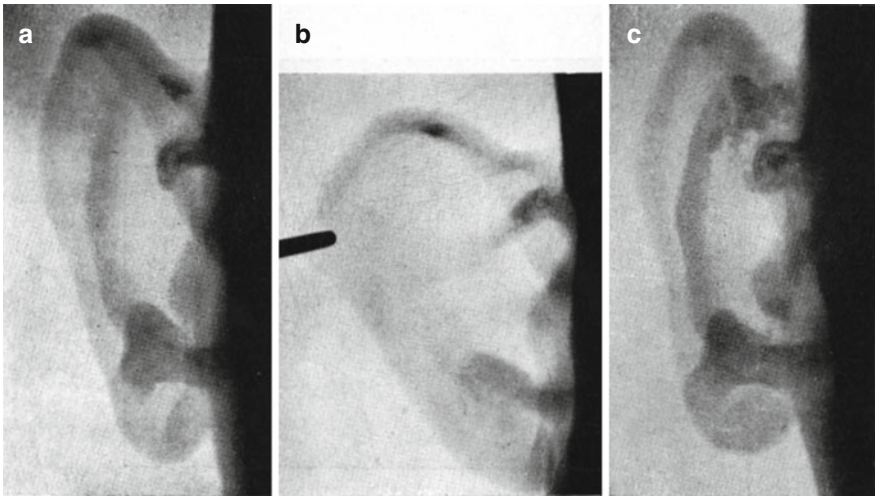

Fig. 17.3 (**a**–**c**) X-rays of the auricles in patients with ochronotic arthropathy

Colour changes were not visible on the cartilage of the external auditory meatus. There was relatively little cerumen in all our patients. Cerumen was dark blue and glossy, and homogentisic acid could be detected in it, as reported in the literature. In one of our patients who had ear frostbites in medical history, dry squamous eczema was present on the skin of the auditory meatus.

None of our patients had normal colour tympanic membranes. In the majority of the cases, it seemed to be darker with a bluish shade in sunlight as well as artificial light. It was opaque, often sunken, with atypical or missing reflex. In the majority of the cases, calcium incrustations were visible on tympanic membranes. In five patients, typical dark brown pigmentations were observed in areas adjacent to the umbo (Fig. 17.4). We did not find any defects or scars on the tympanic membranes of our patients. We have not found any reports in the previous literature of hearing impairment in alkaptonuric ochronosis. However, in our institute, we have performed otologic examination of 48 patients with alkaptonuria, 17 of whom had manifest ochronosis. In patients with alkaptonuria without signs of ochronosis, there was no apparent hearing impairment. These patients also did not have any joint problems. However, out of 17 patients with visible ochronosis, 7 patients reported deterioration in their hearing and 3 patients suffered from tinnitus. Audiometry showed lower hearing acuity in other patients. In total 12 patients out of 17 with ochronosis had hearing impairment. None of these patients had otitis media in the past. Onset of hearing impairment was not sudden in any case; it was insidious. In the majority of the cases, hearing impairment corresponded to mixed hypacusis with more pronounced affection of sound-perceptive apparatus. In eight of our patients, hearing acuity was 2–4 m for whispering (Fig. 17.5a, b). One of these patients was employed at a shooting range, and he attributed hypacusis to acoustic trauma (Fig. 17.5c). The sister of this patient suffered from deaf mutism.

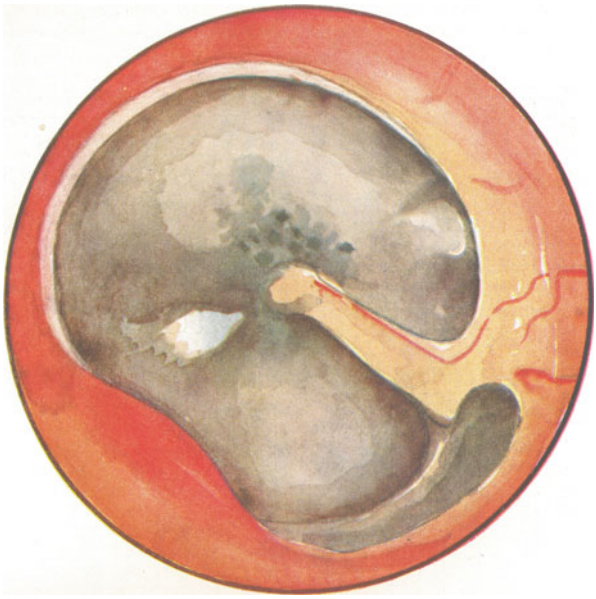

Fig. 17.4 Colour changes on the tympanic membrane of a patient with ochronotic arthropathy

One 57-year-old patient mentioned in his medical history that his grandfather had been deaf and that his brother had deformed auricles. He was suffering from tinnitus in the right ear from the age of 30 and in the left ear from the age of 38. His hearing has insidiously deteriorated, and currently he could only hear loud speech close to the concha. He has been followed by the specialist due to hearing impairment, while the principal disease was not recognised. Therapy was not successful and the Rinne test was negative. The condition was concluded as paracusis. X-ray showed normal pneumatisation. The patient suffered from mixed hypacusis, predominantly the conductive type. The clinical manifestation resembled otosclerosis (Fig. 17.5d).

It can be concluded that if ochronotic changes occur in the course of alkaptonuria, disorder of the ear will also occur in the external, middle and internal ear. Changes of the external ear are typical and characteristic (auricle, cerumen), while the changes in middle and internal ear are variable and probably depend on the extent and localization of the affection caused by harmful effects of homogentisic acid. Injury of the acoustic nerve is typical, and middle ear disorders are also frequent. Otosclerosis can also develop. It seems that the ear of alkaptonuric patients is especially sensitive to injuries in general as well as to acoustic trauma.

An otorhinolaryngologist has to be aware about the nature and external signs of ochronosis that is not as seldom as it was generally presented in the past, in order to contribute to early diagnosis of this disease. We have diagnosed alkaptonuric ochronosis on the basis of ear signs in a patient who came to our clinic due to hearing impairment.

By more detailed analysis of clinical symptoms and their comparison with pathohistological findings, it may be possible to explain pathogenesis of some forms of hypacusis.

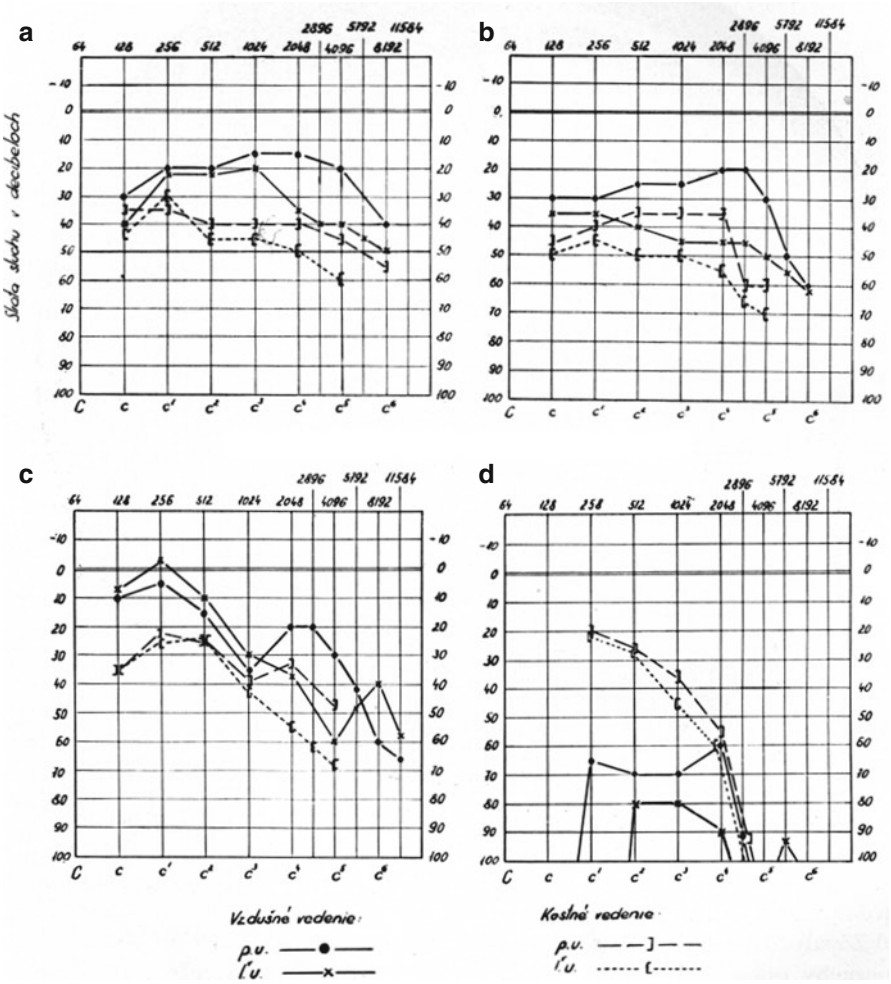

Fig. 17.5 (**a**–**d**) Audiograms of the patients with ochronotic arthropathy

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Manifestations of Alkaptonuria and Ochronosis in Visceral Organs

18

Tibor Urbánek and Jozef Rovenský

Content

Ochronotic pigment is also deposited in visceral organs. In the cardiovascular system, the myocardium, heart valves and blood vessels are involved (Figs. 18.1, 18.2, 18.3, 18.4, 18.5a, b, 18.6, 18.7, 18.8a, b, 18.9, 18.10 and 18.11a, b). Detailed analysis of 26 patients did not show statistically significant myocardial disorders, only earlier onset of sclerotic changes in aorta was observed. Urolithiasis was found in more than one half of the patients, and nephropathy cases were rarely observed.

Alkaptonuria frequently results in significant cardiovascular lesions, particularly aortic stenosis. There is also intensified arteriosclerosis, ochronotic nephrosis and the formation of urinary calculi as additional adverse effects of pigment deposition.

Ochronotic pigment deposition in cardiovascular system may be of clinical importance (Brueck et al. 2008). The pigment is distributed in the walls of blood vessels generally and particularly the aorta, arteries of both large and small dimension, arterioles and capillaries as well as in other, relatively collagenous or avascular connective tissues like the pericardium, valve cusps and rings and organising

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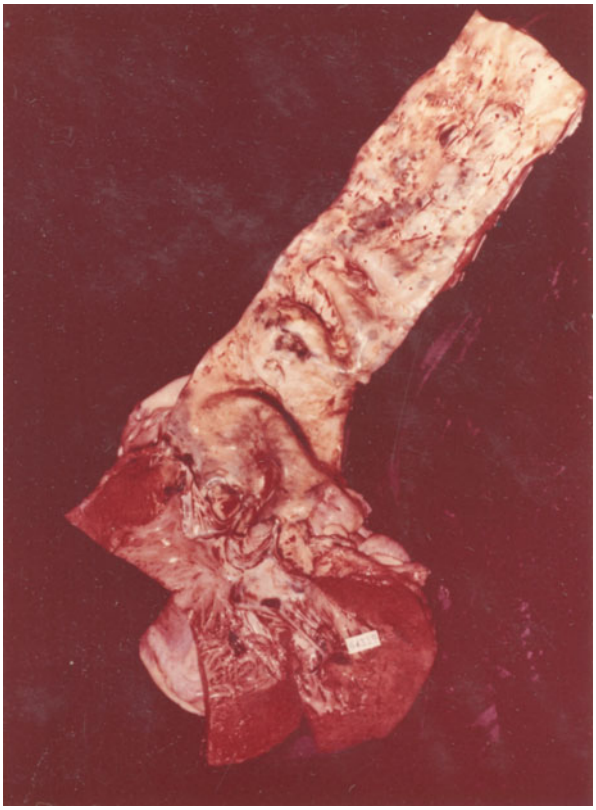

Fig. 18.1 Heart and thoracic aorta. Focal pigmentation on the mitral and aortic valve and sporadically in the subendocardial layer of the myocardium. Ulcerative arteriosclerosis and numerous pigmented foci, especially at the places of atherosclerotic plaques in the aorta

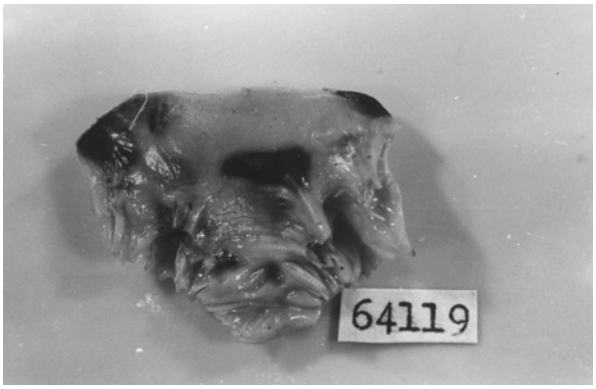

Fig. 18.2 Aortic cusp of the mitral valve. View from below. Pigmentation in insertion part and at the place of the origin of heart strings

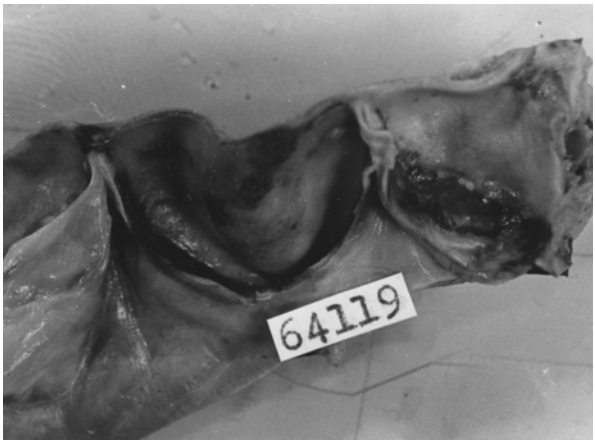

Fig. 18.3 Semilunar valves of the aorta. Considerable pigmentation and calcification of insertion part became more pronounced after the removal of valves

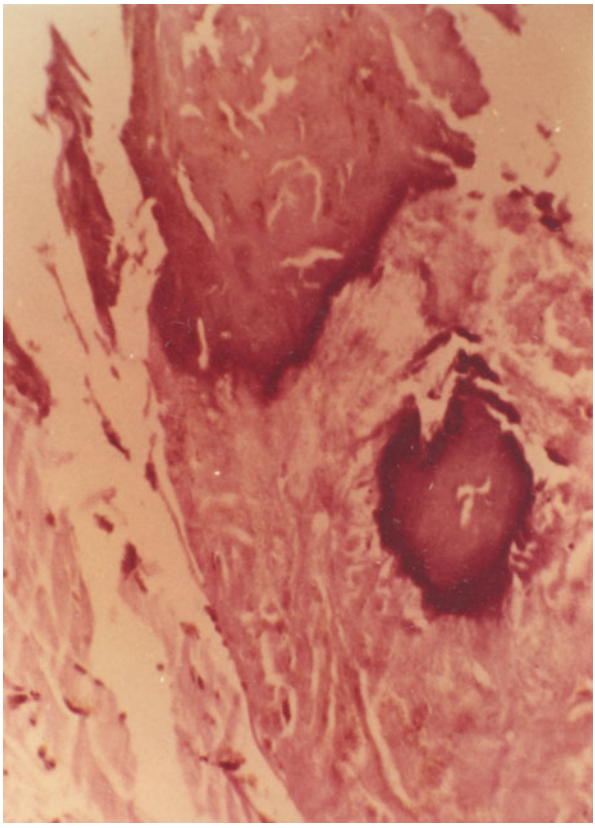

Fig. 18.4 Extensive calcification and pigmentation in basal part of the aortic valve (haematoxylin eosin staining, 260-fold magnification)

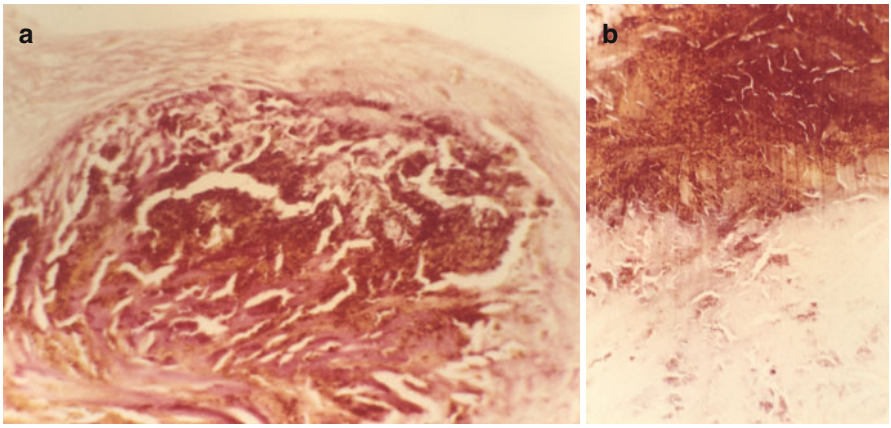

Fig. 18.5 (**a**, **b**) Massive pigmentation in the fibrous annulus of the aortic valve (haematoxylin eosin staining, 130-fold magnification)

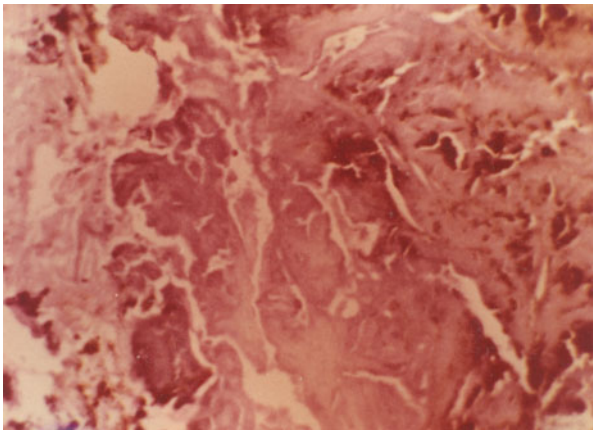

Fig. 18.6 Large calcification in the pigmented area of the fibrous annulus of the aortic valve (haematoxylin eosin staining, 130-fold magnification)

myocardial infarctions (Figs. 18.1, 18.2, 18.3, 18.4, 18.5a, b, 18.6, 18.7, 18.8a, b, 18.9, 18.10 and 18.11a, b). In the walls of the larger arteries, the pigment deposits can be associated with accentuated arteriosclerosis and in the heart valves with calcification, which may result in aortic stenosis (Concistre et al. 2011).

Changes in the urinary tract in ochronosis result from the deposition of the pigment in the branches of renal arteries and arteriosclerosis as well as from the presence of pigment-containing casts within renal tubules, pigmentation of the bladder mucosa, formation of pigmented calculi in the prostate and pigment deposition in

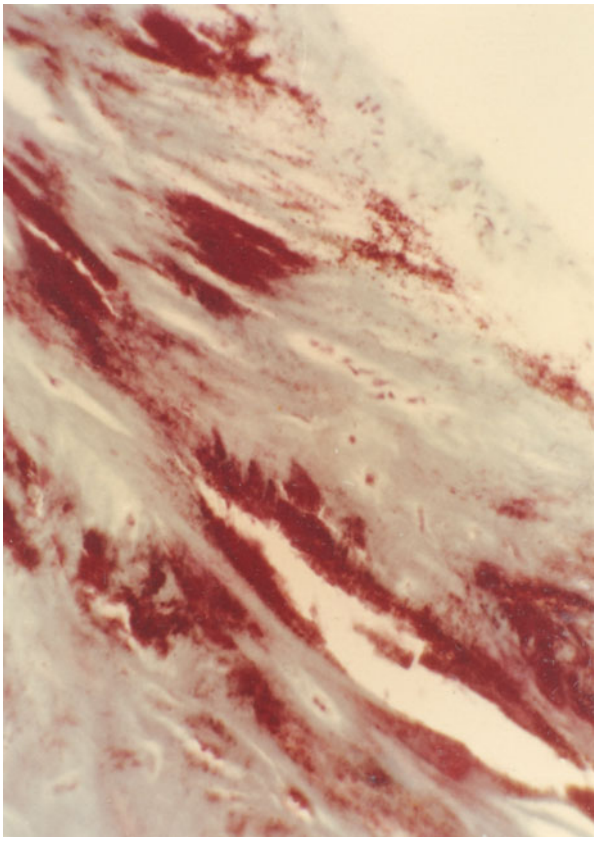

Fig. 18.7 Pigment masses in the fibrous annulus of the mitral valve (trichrome green staining, 130-fold magnification)

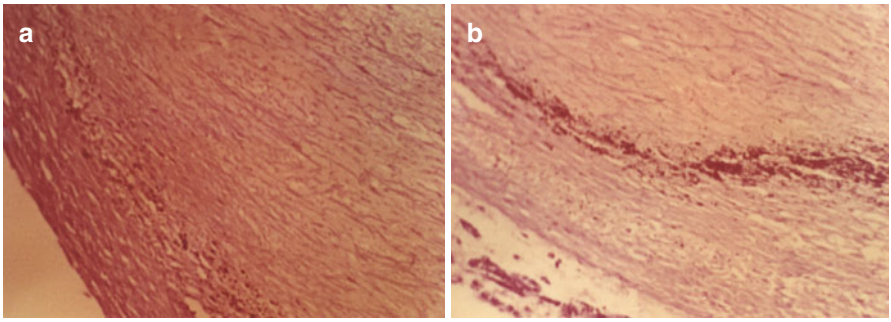

Fig. 18.8 (**a**) Pigment granules in the area of the lamina elastica interna of the coronary artery. (**b**) Marked pigment deposition in the area adjacent to atherosclerotic plaque (cresyl violet staining, 260-fold magnification)

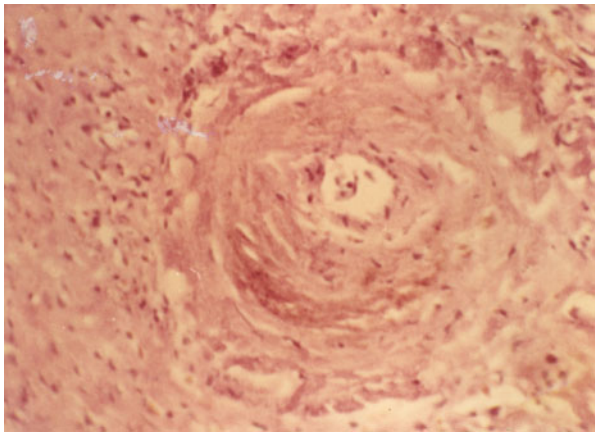

Fig. 18.9 Sclerotic stenosis of the coronary arteriole with pigment deposits in the wall. Fibrosis and pigmentation of adjacent areas (haematoxylin eosin staining, 260-fold magnification)

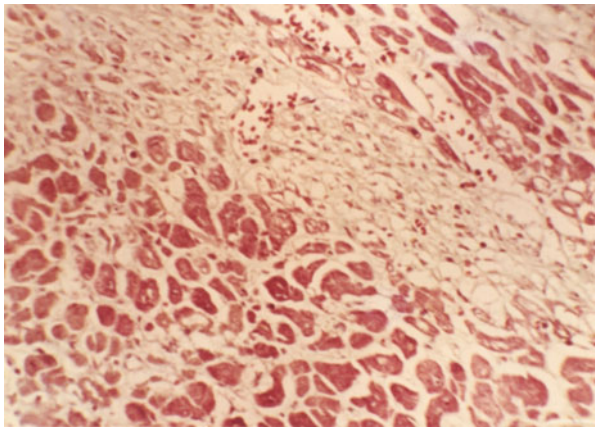

Fig. 18.10 Fragmentation, vacuolar degeneration and focal fibrosis in the myocardium (trichrome green staining, 160-fold magnification)

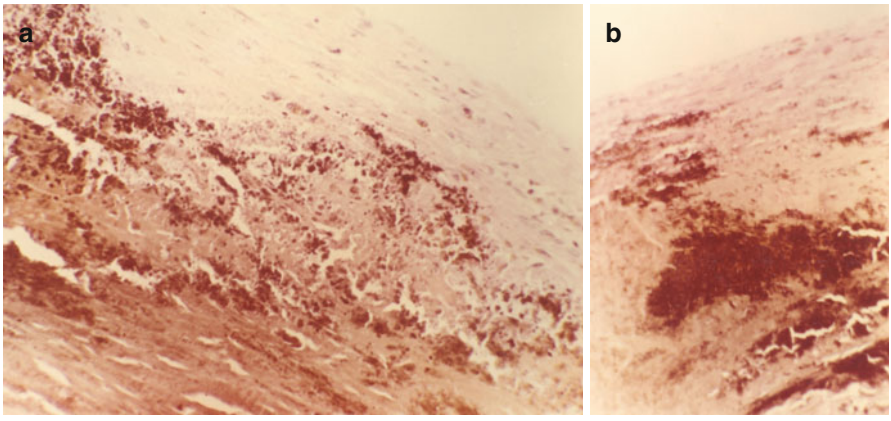

Fig. 18.11 (**a**, **b**) Massive pigmentation of the aortic media in the area of atherosclerotic plaque (haematoxylin eosin staining, 130-fold magnification)

seminal vesicles (Strimer and Morin 1977; Sener 1992). On the other hand, renal insufficiency (due to any reason) causes rapid progression of ochronosis, since renal tubular secretion plays a critical role in eliminating homogentisic acid from the body (Introne et al. 2002).

In the central nervous system, diffuse purple pigmentation was found on the dura mater as well as in the walls of the dural sinuses (Liu and Prayson 2001). The leptomeninges were not pigmented, and the arteries of the Willis circle were markedly less pigmented as might be expected according to their size. Pigmentation was also found in areas of encephalomalacia in the cerebral hemispheres and cerebellum. Pigmented foci of the pineal gland and pituitary body can be found as well (Lichtenstein and Kaplan 1954).

The liver is also affected in alkaptonuria. A patient with repeated occurrence of intrahepatic gallstones probably due to the accumulation of ochronotic pigment was reported in the literature (Bülow and Rosenberg 2009). Another paper presented a patient with ochronotic arthropathy, in whom alkaptonuria resolved and the progressive joint involvement stabilised after liver transplantation for an unrelated problem (hepatitis B-related cirrhosis) (Kobak et al. 2005), confirming that the liver is the main site of homogentisic acid production in alkaptonuria.

The presence of endocrinopathies is not a typical finding in patients with AKU. An association of AKU with diabetes has been probably the most frequent reported in the literature. As early as in 1954, Lichtenstein and Kaplan described an AKU case, in which a pigment deposition was found in pancreatic islets and sinusoidal endothelial cells of the pancreas (Lichtenstein and Kaplan 1954). However, the report does not specifically mention a presence of diabetes in the patient's history. However, the authors also speculated about possible functional consequences of the pigment deposition within the pancreas. Interestingly, Lichtenstein and Kaplan also advised in their paper about frequent diabetes misdiagnosis in AKU patients due to interference of urinary alkaptons with chemical (oxidation-reduction) method of glucose measurement. Currently, this method is not routinely used to measure glucose anymore. In a more recent paper, Introne and her co-workers described an AKU patient with a history of insulin-dependent diabetes and resultant nephropathy (Introne et al. 2002). Recently, another case of a diabetic AKU patient was reported (Naharci et al. 2010). This case report described a 69-year-old woman with diabetes mellitus, ochronosis, depression and chronic pain. Associated comorbidities including type 2 diabetes increase the chance of renal compromise in the perioperative period of AKU patients (Pandey et al. 2011). In conclusion, the association of AKU and diabetes appears to be coincidental. There are no available data supporting the hypothesis of Lichtenstein and Kaplan on functional consequences of ochronotic deposits in the pancreas.

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Manifestations of Alkaptonuria and Ochronosis in the Respiratory Tract

19

Tibor Urbánek and Jozef Rovenský

Content

The patho-anatomical picture of ochronosis in the respiratory tract has been well known for a long time from autopsy and histological findings, whereas the clinical symptoms in this organ system have not been sufficiently observed or described. The reasons were few symptoms and problems with examination of the lower respiratory tract. Therefore, we focused on examining the lower respiratory tract in patients with manifest alkaptonuric ochronosis assuming that the clinical symptoms should be more frequent. Although we have examined only four patients until now, our assumption was correct and fulfilled expectations.

In the upper part of the respiratory tract, there is only a small extent of the cartilaginous skeleton which is limited only to mobile parts of the nose; ochronosis signs were described only rarely, probably because they are asymptomatic and in general context insignificant. Except the nose colouration, the female patient did not have any other signs and symptoms, so this phenomenon could only be used for diagnostic purposes. In the future, it should not be useless to focus more attention to functional examination of olfaction in patients with advanced alkaptonuria. In our four patients, we have not seen marked colouration of nasal cartilages in spite of repeated

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examinations of one patient, manifesting with green fur on the tongue. None of the patients complained of olfactory disorders; however, detailed olfactory examination was not performed.

Clinical picture of alkaptonuria and ochronosis of the lower respiratory tract is much more diverse (Fig. 19.1a, b). The cartilaginous skeleton is much more extensive, and it reaches from the epiglottis to bronchioles. Only the part that is

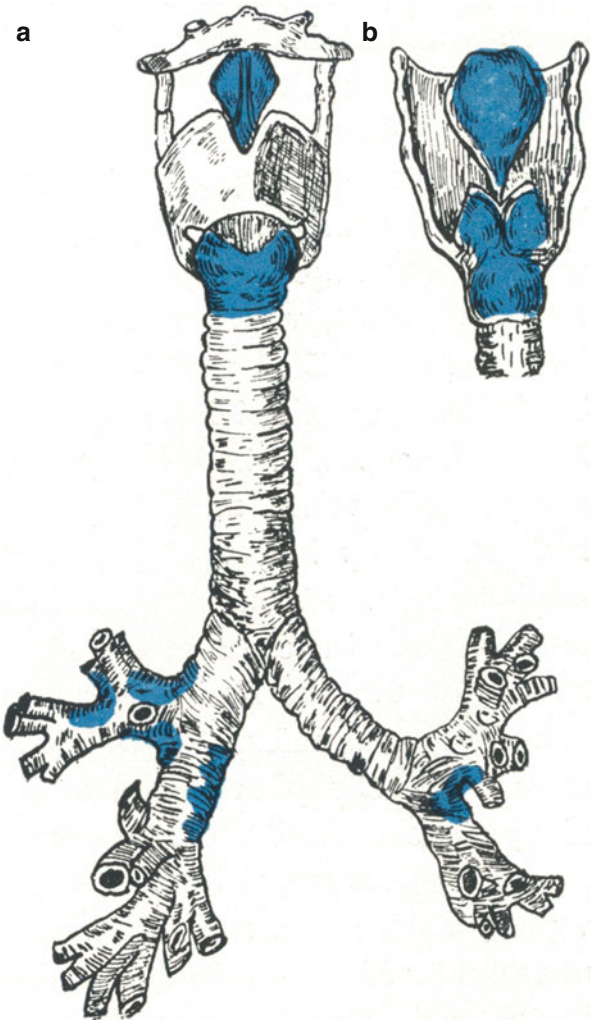

Fig. 19.1 Ochronosis and its location in the cartilaginous skeleton of the lower respiratory tract: (**a**) The cartilaginous skeleton from the front side – the places with considerable ochronotic colouration are marked with blue colour. (**b**) The same changes on larynx rear side. Pigmentation was not apparent at other places of the cartilaginous skeleton, but it does not mean that it could not be present there; it only could not be demonstrated yet

accessible to sight either directly or indirectly, i.e. by laryngoscope or bronchoscope, can be examined. It has to be mentioned here that in the cartilaginous system of the lower respiratory tract in its upper part, i.e. in the larynx, there is also the cricoarytenoid joint. This anatomical note is mentioned because of arthrotropic properties of alkaptonuria. Besides it, the cartilaginous system of the lower respiratory tract has several places that are more functionally strained, e.g. line of division of main, lobar and segmental bronchi. This anatomical note also applies to ochronosis that occurs at certain predilection places.

In literary sources, we found the case of Gross and Allard (1907) who found, during autopsy, typical colouration of cartilages of the larynx, trachea and bronchial tree in alkaptonuric patient who died of sepsis. Besides the colouration of auricles, they found dark-coloured, brittle laryngeal cartilages similar to burnt wood. Histologically, it was confirmed that the changes were caused by deposited pigment in cartilaginous tissue. Ochronosis was also histologically confirmed in the trachea and bronchi. We quote the findings in the trachea: 'Dark brown pigment is found in perichondral tissue as well as in connective tissue, mostly in the form of narrow bands and rarely accumulated in larger conglomerates'. Excerpt from lung histology: 'Small bronchopneumonic foci sporadically, oedema sporadically. Granules of dark pigment around bronchi can be found at some places'. It means that the pigment was found around very small bronchi going to the lung parenchyma. All tracheal and bronchial tree rings macroscopically had black colouration. In this case, ochronosis completely affected the cartilaginous skeleton of the lower respiratory tract as well as larynx. Histological examination was not performed either from the larynx or larger bronchi.

It can be seen from autopsy findings of Švejda's case (1945) that ochronosis can affect the whole cartilaginous system of the lower respiratory tract. We have always seen inhomogeneous colouration of the respiratory tract in the larynx as well as in the trachea and bronchi. We found colouration of cartilaginous rings in the bronchial tree anywhere in the visual field, but the most intense colouration was always present at the places of lobar lines of division in segmental bronchi. It is probable that these changes are repeated in further peripheral parts, to sub-segments, etc. (Fig. 19.2a, b).

Naturally, intensity of colouration is various, and thus, the quality or colouration tone partially changes from slight bluish tinge to deep green-blue colour. Pigment patches never have sharp margins, they are not well demarcated, and they fade away in surrounding area. If they have a small intensity, they are exclusively limited to cartilaginous rings. In case of deep colouration, they also overlap to intercartilaginous connective tissue membranes. Even in case of intense pigmentation, we have never seen colouration of the paries membranaceus of the trachea and main bronchi. These characteristic properties of ochronotic pigmentation have to be known to distinguish from other pigmentations that can be encountered on mucosa and submucous tissues in the respiratory and gastrointestinal tract. We can see physiologic as well as pathological pigmentations in the bronchial tree. Pathological pigmentations can be seen in mercury and bismuth poisoning, haemosiderosis, Addison's disease, pernicious anaemia, melanosarcoma, malaria, icterus, etc. Some

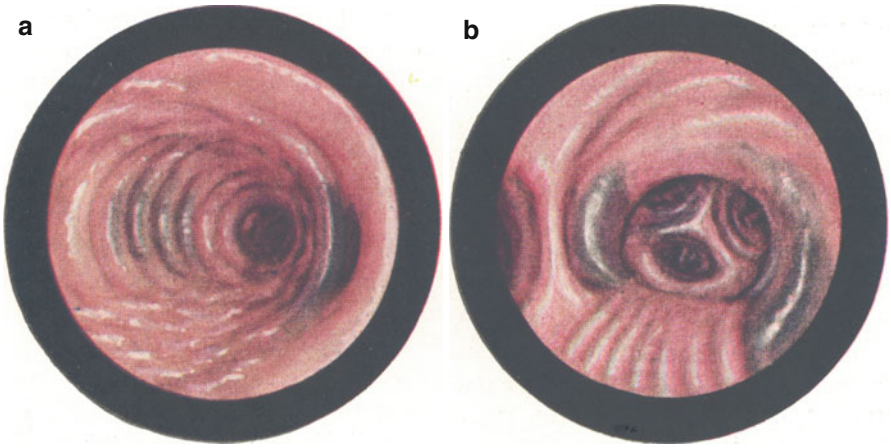

Fig. 19.2 Ochronosis in the bronchial tree. (**a**) The right main bronchus with the origin of the upper lobar bronchus. Colouration at the division line of upper and connecting part and on internal wall of the main bronchus. (**b**) The right upper bronchus with segmental trifurcation and several subsegmental bronchi. Colouration is usually located at division lines

of them can be recognised easily; other ones can be hard to be diagnosed. A lot of attention has to be paid to distinguish ochronosis from anthracosis. Anthracotic colouration is always more intense, black, well demarcated and located mostly outside cartilaginous rings. Typical location is in lymphatic nodes, blood vessels and their adjacent area.

We have not seen deforming zones on the cartilaginous skeleton of the respiratory tract, and we can rule them out on the basis of normal respiratory and pulse activity of the bronchial tree.

From the technical point of view, it has to be mentioned that ochronotic colouration of small intensity can easily be missed; on the other hand, intense colouration can be confused with other types of pigmentation. Therefore, all places of the trachea and bronchial tree have to be examined not only in tangential view but also in rectangular view because in this way the end of bronchoscope leans against the mucous membrane which is then pressed and becomes thinner. This results in better visualisation of coloured cartilaginous base. It is also necessary to change light intensity because yellow tone of weak light hides fine tinges of colouration that are characteristic for various kinds of pigmentations. From the clinical point of view, ochronosis of laryngeal cartilages does not affect the whole skeleton. Sometimes we found colouration of the epiglottis and arytenoid cartilages; sometimes the epiglottis colouration was not found. Detection of ochronosis in the larynx depends on the type of examination. Indirect laryngoscopy reveals only colouration of certain intensity limited solely to the epiglottis. We can never see pigmentation of arytenoid cartilages, even in manifest forms with intense pigmentation. It is caused by a thick layer of soft tissue that covers the cartilages. Colouration of these cartilages can only be detected by direct laryngoscopy when we press the soft tissues and make

them thinner to better visualise coloured base. Ochronosis of the thyroid and cricoid cartilage can be detected only by surgical methods, but according to autopsy findings, we can assume involvement of other parts of the larynx cartilaginous skeleton, if the epiglottis and arytenoid cartilages are affected by ochronosis.

Cricoarytenoid alkaptonuric arthropathy requires presence of ochronosis. The cartilage reacts to deposited pigment by secondary chronic inflammatory process. Cartilaginous tissue disintegrates due to aseptic inflammation with the subsequent proliferation of synovium and neoformation of blood vessels in it. Deforming changes arise in the joints, and joint ankylosis occurs after cartilage involution.

From the clinical point of view, these pathological-anatomical changes are firstly manifested by cartilage colouration that we commonly do not detect by laryngoscopy due to the above-mentioned reasons. Inflammatory process on cartilages results in clinical bulging and oedema of arytenoid eminence – the picture we can see in perichondritis or arthritis of other origin. Recognition of the disease in this stage is very difficult or even impossible. Functional disorders become marked after the acute changes disappear, i.e. limited movement of arytenoid eminences and vocal cords that finally results in complete immobility and fixation in paramedial position. If the changes appear on both sides and vocal cords remain in paramedial position, we can see the same picture as in bilateral paralysis of laryngeal nerve.

Ochronosis of arytenoid cartilages itself does not cause any subjective problems. However, arthropathy manifests with pain during speech and especially during swallowing. Dyspnoea of variable intensity can also be present, depending on the degree of inflammatory changes of larynx soft tissues. Ankylosis of the joints causing paramedial fixation of vocal cords always leads to severe dyspnoea that will finally require tracheotomy.

Recognition of alkaptonuric arthropathy and ankylosis is very difficult in each stage, and only direct laryngoscopy enables it. This examination can detect ochronotic colouration of cartilages. In the stage of inflammation, it can easily be confused with any kind of inflammation but also with retrocricoid tumour – carcinoma. We considered our case to be a tumour for a long time. The diagnostic aid, similarly as in the trachea and bronchi, can be the X-ray examination of the larynx and bronchial tree where we can detect demarcated opacities caused by salts deposited in damaged cartilage (like in auricle ochronosis). Ossification pattern of the laryngeal skeleton is more intense, more extensive and irregular, and we can observe it at the places that are usually not affected.

The treatment of alkaptonuria and ochronosis in the respiratory tract is primarily causal. According to local manifestations, local palliative treatment is sometimes required. Dyspnoea caused by paramedial position of vocal cords can only be removed by tracheotomy. If a patient does not want to have the cannula permanently, we can help him get rid of it by laterofixation of the vocal cord. With regard to tissue condition based on regressive changes and disintegration of cartilaginous tissue, the procedure can have uncertain results. Therefore, we have to assess the disease from prognostic point of view as serious one because in a certain stage it can be life threatening. It can also lead to permanent disability because the patient has to have cannula throughout life.

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Pathological Anatomy and Histology of Ochronosis

20

James A. Gallagher, Štefan Kopecký, Svetoslav Štvrtina, and Jozef Rovenský

Contents

20.1 General Part

The classical description of the pathological-anatomical changes in ochronosis was made by Virchow (1866). Since then, many authors have contributed to the literature (Sternberg 1934) Oppenheimer and Kline (1922) (Coodely and Greco 1950). The majority of authors have suggested that deposition of ochronotic pigment takes place most frequently in poorly vascularised cartilage and that this increases with

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age (Abbot and James 1950; Bödeeker 1859; Bauer and Kienböck 1929; Söderbergh 1915; Gross and Allard 1907; Uebermuth 1928 and others). Deposition of ochronotic pigment has been most widely observed in intervertebral discs and perichondrium. Pigmentation is seen less frequently in the periosteum and lower still in tendons and other soft tissues subject to age-related degeneration. Ochronosis of articular cartilage results in and increase in stiffness and loss of flexibility. Cartilage becomes brittle, cracks appear, and then it breaks up into fragments similarly to avascular necrobiosis of epiphyses of long bones. Uebermuth (1928) reported that even minimal loading led to partial or complete detachment of fragments of articular cartilages.

Švejda (1945) reported a case of ochronosis identified at autopsy without previous clinical diagnosis. In this study, ochronotic pigment was found in blood vessel walls, less in cartilages and perichondrium. Cartilage was affected by large regressive changes, and there was destruction of bony tissue in the femur Sternberg (1934).

Galdstone et al. (1952) made a more detailed report on autopsy of two ochronotic patients. In the first patient, they found pigment deposits in the endocardium and heart valves as well as changes to the intervertebral discs, which were narrowed and calcified, with dark brown colouration. Sternoclavicular and costochondral cartilages were similarly affected. Ochronotic changes in the prostate were especially marked. The second patient had characteristic deposits of ochronotic pigment in the calvarium, dura mater, larynx and large blood vessels. The vertebral bodies were atrophic and fused sporadically, and discs were dislocated and had dark colouration.

Lichtenstein and his co-worker Kaplan (1954) made a detailed description of pathological-anatomical and histological changes of ochronosis. They observed that rib cartilages, the anterior longitudinal ligament and ventral part of sacral vertebrae were pigmented. They identified ochronotic pigment concentrated centrally on intervertebral discs. Many discs had severe degeneration, and even completely preserved discs were impregnated by the pigment. There was a tendency for protrusion of discs and associated osteoarthrosis, and contiguous vertebral bodies had fused. The knee joints were considerably pigmented, especially on capsule and tendons. Cartilaginous fragments were found in intra-articular space. The cartilaginous ends of adjacent bones were ulcerated and had black colouration. Synovial membranes were thickened and deeply infiltrated by the pigment. Symphyses were also considerably pigmented, and nodular pigmented osteoarthrotic protrusions were present.

Histological investigation revealed that the pigment appeared granular, rather than crystalline colour. Deposition of ochronotic pigment in the wrist was accompanied by a discrete inflammatory reaction, and giant cells could be found sporadically, especially near detached cartilaginous fragments.

Observations of ochronotic osteoarthrosis have frequently been made at joint surgery. Kolaczek (1910) reported dark pigmentations of articular cartilage during surgery on a knee with a fungal infection. Procházka and Hněvkovský (1953) identified ochronosis during a knee biopsy on a 62-year-old female patient. They observed green colouration and loss of cartilage on the patella and on the condyles. These authors as well as Červeňanský during the lecture at the Czechoslovak

Physiatrie Society in 1953 emphasised that most severe ochronotic changes are not located on cartilages but in subchondral spaces and especially on the tendons. In his lecture, Červeňanský et al. showed a colour film on the case of a 70-year-old female patient who had previously undergone a biopsy of the hip joint with epiphyseal resection and subsequent osacryl arthroplasty and also underwent a biopsy of the knee joint. Contrary to the majority of the authors, they reported that ochronotic pigment does not deposit most frequently in poorly vascularised and degenerating cartilage but that ochronotic pigment was massively deposited in ligaments, periarticular tissue and especially at the places of insertions of tendons and capsule. We observed similar findings during a surgical revision of the right hip and knee joints performed in one of our older patients.

During surgery of the right hip joint, a 20 cm long semi-arch incision was made from the spina iliaca ventralis under and behind the greater trochanter. After the discission of the fascia lata just under the trochanter and incision of aponeurosis of medial margin of gluteal muscles, isolation of internal margin of gluteal muscles from the tensor fasciae latae was firstly carried out. After isolating and preparing the ventral part of the capsule, the following situation was found. After exposing the capsule, dark blue patches successively appear, firstly in superficial layers of the capsule as well as on adjacent muscle fasciae. These dark blue patches had the character of spots, like after spilling ink. It was apparent that these were clusters of ochronotic pigment that were most marked on supraacetabular insertion of the rectus femoris muscle. The supraacetabular origin of this muscle had the shape of an intensely dark blue incrusted button. There were also marked ochronotic changes on acetabular and on trochanteric insertion of the capsule. The capsule was cut, and as it firmly adhered to the femoral neck in the whole extent, it was gradually removed together with accumulated lumps of ochronotic tissue. After removing the ventral part of the fibrous shrunken capsule, it was apparent that these ochronotic pigment formations stood out from the neck of the compact bone like osteoid excrescences. One such formation in the middle of the medial part of the trochanteric crest was especially massive, and it looked like a crystalline deposit or exostosis deeply implanted in the spongy bone of the neck and trochanteric mass.

After the adduction and extrarotation of the femur, the lateral margin of the femur head was visible. It had ochronotic colouration in the form of transversely located black stripe in the laterodistal quadrant. As the luxation of the femur head was not successful, we performed its resection and extraction. Macroscopic examination of the extracted femur head was of medium size, and its surface was almost nearly completely free of cartilage except for remains of atrophic cartilage in the form of rare islets. The whole femur head was completely matt without any gloss. In the lateral quadrant, there was one transverse line, not completely continuous, drawn like by the carbon pencil of 2.5 cm size. Another similar semi-arch shaped line, 2 cm long, was present in the medial quadrant of the femur head. The subchondral part of the femur head as well as spongious bone was sclerotic with an unusual appearance like hardened cement. Longitudinal incision of the head of the femur revealed that pigment extended to 4–6 cm in subchondral direction.

The medullary part of the femur head was also sclerotic, and there was a wedge-shaped focus in its central part that has pink tinge, whereas the other bone substance had grey-yellow tinge. In the acetabulum, there were only three small flat regions of ochronotic pigmentations in close vicinity to the origin of the ligamentum teres femoris. The acetabular cartilage was also atrophic and sclerotic, but it still had some gloss.

After the resection of the femur head, remains of the neck were adjusted for osacrylic prosthesis that was firmly fixed, and the hip was reduced. Post-operative course was uncomplicated.

The second bioptic revision was performed on March 26, 1953. A 14 cm long longitudinal incision at the medial part of the right knee joint was done. After the discission of the hypodermis and superficial fascia, we could observe dark blue patches, especially on the internal side of the retinaculum. The ligamentum patellae proprium and insertion tendon of the quadriceps femoris muscle were markedly coloured by ochronotic pigment. After the preparation of the vastus medialis muscle and capsule discission that were open completely, we found the following situation after arthrotomy. Synovial villi in the medial and lateral recess or over the recess had dark brown colour. The synovial membrane of the capsule was imbibed by blue patches. The internal femoral condyle was markedly changed. Its cartilaginous part and partially osseal part were continuously covered by dark blue pigment deposition that was firmly fixed directly in cartilage and did not damage it at all from macroscopic point of view. Superficial cartilage seemed to be completely intact. There were similar changes on the lateral femoral condyle, but not so marked.

The tendon of the quadriceps femoris muscle and some deeper tendons were completely hardened and impregnated by dark blue ochronotic pigment in their insertion part. Basal part of these tendon insertions was firmly fixed to the upper lateral margin of the patella and completely blended with it. Tendon insertions looked like exostoses growing from the patella. Part of the tendon over the hardened place was excised in order to perform histological examination.

As we mentioned before, the ligamentum patellae proprium was markedly impregnated with ochronotic pigment. After longitudinal discission of this ligament, fascicles completely separated, and basically the whole tendon was completely necrotic due to ochronotic pigment imbibition. The patella was excided subperiosteally.

The macroscopic appearance of the patella was as follows: the articular facet was thickened and bended, markedly rugged. The whole patella had irregular shape. At the peripheral part of the articular facet, there was nearly completely continuous pigment stripe of 3–6 mm width. Colouration of this stripe was partially more intense. Ochronotic pigment deposits with completely black colour were also present in superficial layer at the place of periosteal reaction where the ligament of the quadriceps femoris muscle was inserted. After the longitudinal incision of the patella, we can see that the pigment reaches only to the subchondral area and does not reach the deeper layer of the spongious bone. Reconstruction and suture of capsule and quadriceps femoris tendon were performed.

20.2 Histological Picture

The head of the femur, parts of the joint capsule and the insertion of tendons contain foci of black pigment which are macroscopically visible foci. They were fixed by formaldehyde. Sections, 10-15 μm thick, prepared from the area of these dark foci and their adjacent area, located in stiff connective tissue of tendons and joint capsule as well as tangential sections from thin, hardly recognisable cartilaginous coating of the femur head, were stained by haematoxylin eosin. The histological images of the connective tissue of tendons and fibrous capsule are displayed in Fig. 20.1a–g.

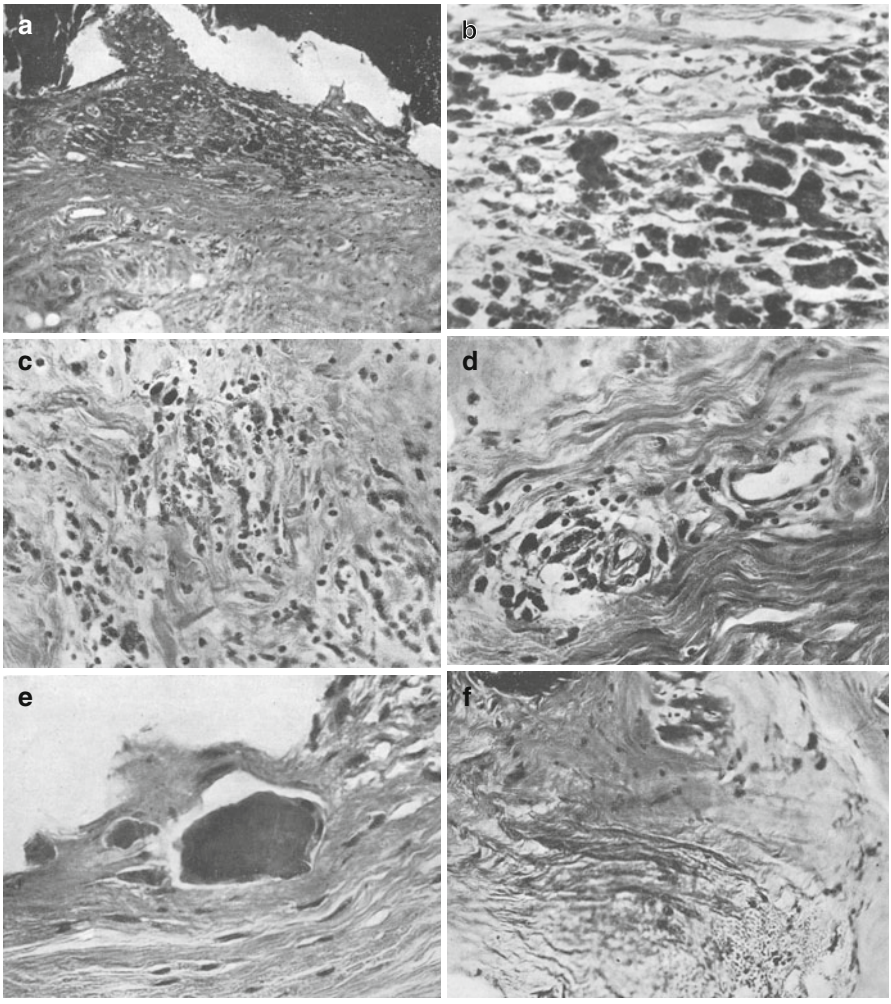

Fig. 20.1 Ochronosis in joint tissues. Histological images of the connective tissue from the tendons and fibrous capsule of a patient with AKU showing deposition of ochronotic pigment (**a–g**). Photographs of the open hip joint (**h**), knee joint (**i**), head of femur (**j**) and patella (**k**)

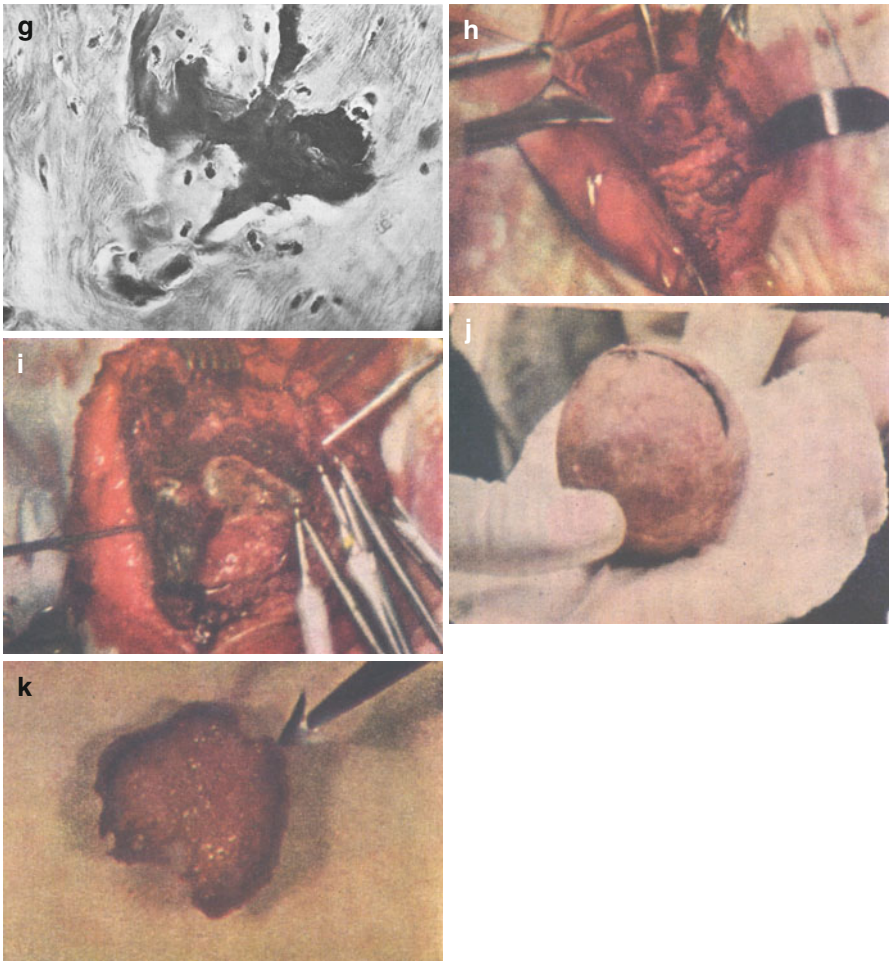

Fig. 20.1 (continued)

The tough connective tissue of tendons and fibrous capsule contains dark black, macroscopically recognisable foci of pigment of various sizes, from large pigmented areas visible macroscopically to individual pigment granules visible only by microscope. During the processing, the pigment accumulated in large foci falls apart, and during the slicing, it gets to marginal areas adjacent to tough connective tissue. Tough connective tissue around the focus has a characteristic concentric arrangement, and fibres run around pigment granule in several layers. At many places, the tissue loses its regular pattern of collagen fibres, and irregular spaces are filled with fibrous cells with hypertrophic cytoplasm between them or their fascicles arise. The cell bodies are of considerable size, with polygonal or oval shape, and it contains an oval nucleus with well-stainable chromatin structure. The cytoplasm of these cells contains various amounts of brown pigment granules from a few pieces

up to large amounts filling the whole cell body and covering the nucleus. It can be seen that cells overfilled with pigment decompose, pigment granules are released from them, and they gradually form to freely deposited bigger or smaller granules and lumps of pigment mass between the fibres (Fig. 20.1a–c).

There are no signs of inflammatory reaction in the connective tissue around such pigment foci. However, adjacent connective tissue is relatively well vascularised, and small blood vessels (small arteries, arterioles and veins) can be seen in divided and loose connective tissue. Adventitia of these vessels contains large amount of polygonal and oval cells with larger or smaller amount of brown pigment granules in cytoplasm. The number of these cells varies; there are only a few of them at some places, but in the other places, they form apparent perivascular foci (Fig. 20.1d), or they can even be present independently from blood vessels, especially in thinner interstitial connective tissue. More distant places from pigment foci usually have normal structure of tough connective tissue with typical nuclei of tendon cells.

In some areas, especially under the surface of tendons and capsule, degenerative changes can be observed in areas containing pigmented cells or even large pigment foci (Fig. 20.1e). Groups of well-demarcated connective tissue fibres of various sizes are hyalinised; their fibrillar structure has been lost creating a homogeneous, deeply basophil mass with sporadically included polygonal cells with pigmented cytoplasm. Even in adjacent areas of such hyalinised parts, cells containing pigment granules can be seen in connective tissue. Signs of necrosis and thin fibrillar structures characterised by intense basophilia can be sporadically seen at some places. The thin coating of joint cartilage is incomplete. Degenerate hyaline cartilage with distinctive fibrillar matrix (Fig. 20.1f) and well-demarcated pigment foci of dark brown colour and various sizes (Fig. 20.1g) can be seen in the sections from greyish spots of this coating. These foci are distinctly separated from adjacent area; their margins look like they have been cut, while there are no particular reactive signs in adjacent tissue.

Some specific data (situation from open hip joint can be seen at Fig. 20.1h) have to be pointed out from the surgical report of our 70-year-old patient dated January 24, 1953: supraacetabular insertion of the rectus femoris muscle as well as acetabular and trochanteric insertion of the capsule had marked dark blue colouration. It results from the second surgical report dated June 20, 1953, that the subcutaneous connective tissue of the right knee joint as well as deep muscular fasciae had dark blue colouration. The ligamentum patellae proprium and the whole tendon of the quadriceps femoris muscle were massively impregnated by ochronotic pigment (Fig. 20.1i). On the other hand, the medial femoral condyle was also covered by dark blue pigment, and it impregnated the whole absolutely damaged cartilaginous and bone part of the medial and lateral condyle.

The following changes were found on the femur head and patella during their surgical removal:

Femur head: it did not have any cartilaginous glossy coating except some small remains. The epiphysis surface had grey colouration, and it was dull. In the lateral quadrant of the epiphysis, there was one 2.5 cm long, not a completely continuous line that looked like artificially drawn by carbon pencil (Fig. 20.1j). Not far from it,

there was another 5 cm long arch-like line. The subchondral part and spongy bone were markedly sclerotic. Section through the head showed that these pigment lines reached up only 4–6 mm in subchondral direction.

The articular facet of the patella was bended, rugged and thickened. There was a nearly complete continuous pigment line of 3–6 mm width going through the peripheral part (Fig. 20.1k). At the place where the tendon of the quadriceps femoris muscle goes to the patella, carbon-coloured deposits were visible. After splitting the patella, we could see that pigment line reaches only subchondrally and does not reach deeper layer of the spongy bone. It was a completely identical picture as on resected proximal epiphysis of the femur. It has to be noted that neither in the acetabulum nor in the intra-articular part of the knee joint, no free particles of detached cartilage that would be impregnated by ochronotic pigment were found. Multiple foci of ochronotic pigment are located especially in loose fibrous connective tissue. We also found the location of fibrous tissue cells with pigment granules in perivascular area as well as pigment foci among the fibres of solid fibrous connective tissue. Solid fibrous tissue showed the signs of hyaline degeneration, and it was pigmented. 'Shadows' of chondrocytes can be seen in the mass of pigment indicating that ochronosis occurred by uptake of pigment into cartilage matrix and chondrocytes and/or trapping of chondrocytes in the matrix during the course of pigmentation. The structure of undamaged cartilage areas shows typical senile changes with multiple isogenic groups of cells.

On the basis of the study of clinical material in 26 cases of ochronotic osteoarthrosis, we noticed that ochronotic arthropathy has its specific, incipient as well as developed and especially characteristic constant symptoms of advanced disease. According to these analysed X-ray signs, the disease can be diagnosed with high probability. Except the narrowing, fragmentation and pycnosis of intervertebral discs, the very typical or even specific signs are chondro-osteoid pseudocystic translucencies at the place of insertions of some ligaments that are usually massively impregnated by ochronotic pigment (tendon of the caput longum biceps femoris, tendon of the rectus femoris muscle, vertebral short and long ligaments).

It is interesting that at the place of pigment deposition in tendons, especially if considerable incrustations or even calcifications were present, no calcium concrements were detected by microscopy. On the other hand, X-ray of bone structure showed the most marked signs of condensation with simultaneous patchy translucencies that often looked like pseudocysts. Our observations do not support the currently accepted opinion that imbibition of articular cartilage by homogentisic acid has to result in degenerative or inflammatory process with the subsequent decomposition and calcification. Calcification of tendons and fibrous tissue generally occurs as a consequence of degeneration and necrosis. It is mostly amorphous calcium carbonate and calcium phosphate that is deposited at the margin of degeneration and usually at the place of tendon necrosis.

We still do not know the biochemistry and histochemistry of calcification in relation to ochronotic pigment and especially to alkaline phosphatase and glycogen, and we will study it systematically in the future.

20.3 Conclusions of Histological Findings

1. Ochronotic pigment was not deposited on the whole proximal epiphysis of the femur, but only in a very small amount in the form of two small lines looking like drawn by carbon pencil. The same changes were observed on the patella that was completely deprived not only of original gloss, but the whole cartilage and all its layers were atrophic with subsequent superficial defects like after usuration. Besides the circular narrow band, the patella was not much impregnated by ochronotic pigment as we would expect.
2. On the other hand, we could see again that both femoral condyles had deep dark blue colouration. Macroscopic appearance of the cartilage was normal and seemed to be completely intact. Original gloss, thickness and functional character of normal cartilage were preserved. Only deep dark blue colouration of the cartilage seemed to be pathognomonic.
3. All tendon and fibrous formations located in intra-articular and especially periarticular area were impregnated by the pigment, especially the insertion parts.
4. Where we macroscopically found very advanced degenerative and necrotic changes.

We can say that:

(a) Cartilage is not the only principal substance to which ochronotic pigment binds.
(b) Cartilage can be completely intact according to macroscopic appearance, and simultaneously it can be massively impregnated by the pigment.
(c) Among articular structures, tendons and especially their insertions are most affected by imbibition of ochronotic pigment.

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Picture of Synovial Effusion in Ochronosis

21

Tibor Urbánek and Jozef Rovenský

Content

Knee changes basically have arthrotic character. They differ from genuine osteoarthrosis by earlier onset (average age of 39 years), more rapid progression and larger deformities. Hydrops occurred in 30.4 % of our patients. On the basis of series of examinations, Hüttl et al. (1966) found out that synovial effusion has non-inflammatory, irritation-degenerative character. Effusion is yellowish, and the colour does not change even if it stays in the open air for a longer time, suggesting low concentration of homogentisic acid. From the nosographic point of view, finding of histiocytes with brown-purple to blue-black cytoplasmatic inclusions that probably represent phagocyted ochronotic pigment is significant (Hüttl et al. 1966). Hüttl et al. were the first in the world literature to describe finding of histiocytes with pigment inclusions in cytoplasm as it was pointed out by P. Stiehl and K.M. Kluger from the Institute of Pathology at the Leipzig University (Stiehl and Kluger 1994).

Urine alkalization by 10 % NaOH can be used as a screening method. After adding NaOH to the tube with urine, dark ring forms, and later the whole urine sample becomes darker. Sršeň and Neuwirth (1974) developed a practical method

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suitable for field surveys. Strips of filtration paper impregnated with 40 % solution of NaOH are firstly dried, and after their submerging to urine sample, diagnostic strip stains to various tinges of brown colour within 3–5 min according to the concentration of homogentisic acid in examined urine (Sršeň and Neuwirth 1974).

Chromatographic methods are used for precise detection of homogentisic acid in urine. To assess the amount of excreted homogentisic acid in urine per 24 h that usually ranges from 2 to 6 g in case of normal food intake, quantitative methods like iodometric, colourimetric, etc., are used. Enzymatic method according to Seegmiller et al. (1961) is the most specific. Trnavská (1962) implemented the method of electrophoretic detection of homogentisic acid.

Diagnosis of ochronosis is based on the finding of pigment spots on eye structures, grey to blue colouration of auricles and skin in armpits and X-ray finding of calcified intervertebral discs. In more advanced stages of the disease, irregularly prominent spinous processes in the thoracic and lumbar spine are typical for ochronotic arthropathy, and the finding of pigmented inclusions in synovial effusion cells is specific.

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Rare Complications of Alkaptonuria: Haemolysis and Amyloidosis

22

Richard Imrich, Jozef Rovenský, and Žofia Rádiková

Content

High plasma levels of homogentisic acid in alkaptonuria can be a potentially fatal condition, due to the toxic effects of the homogentisic acid, melanin (soluble and deposited), its intermediates and reactive oxygen side products. Their toxicity increases when antioxidant mechanisms are overwhelmed, and their formation in blood causes haemolysis (Hegedus 2000). However, the relationship between high plasma HGA concentration and haemolysis is still questionable. Alkaptonuria patients develop arthritis and often suffer from other diseases too, including cardiovascular and kidney disease. Fatal alkaptonuria cases are infrequent, and death often results from kidney or cardiac complications. Heng et al. (2010) reported a case of a young patient with alkaptonuria suffering from severe renal failure who developed fatal metabolic acidosis and uncontrollable intravascular haemolysis, which may

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have been caused by rapid and extensive accumulation of HGA and subsequent accumulation of plasma soluble melanins. Decreased serum antioxidative activity has been reported in patients with chronic decreased kidney function. Despite administration of large doses of antioxidant agents and intensive kidney support, haemolysis led to the death of the patient.

The second fatal case of uncontrolled haemolysis in an alkaptonuria patient after kidney transplantation was reported by Bataille et al. (2014). During autopsy, massive deposits of HGA-melanin pigment were found in the kidneys, aortic valve, coronary arteries and lung macrophages and in the liver with centrolobular hepatocyte necrosis.

It is not clear what triggers haemolysis. In the literature, cases of renal failure in alkaptonuria are rare. Chronic renal failure leads to poor HGA clearance, and thus elevated HGA levels which might increase haemolysis risk (Introne et al. 2002). The triggering of acute haemolysis might besides HGA accumulation also need a 'second hit', i.e. oxidation.

Reactive systemic AA amyloidosis is one of the most severe complications of several chronic rheumatic disorders (e.g. rheumatoid arthritis, ankylosing spondylitis). Deposition of amyloid in the synovial membranes of the joint or of the tendon sheaths causes joint symptoms (stiffness, swelling and movement limitation).

Millucci et al. (2012) found that alkaptonuric amyloid was co-localised with HGA-melanin ochronotic pigment. It was suggested that HGA polymer may be involved in amyloid deposition.

The co-localisation of HGA-melanin and amyloid suggests the participation of fluorescent oxidised HGA pigment in the formation of amyloid aggregates and also a link between HGA oxidation and amyloid deposition.

Amyloidosis is a secondary complication of AKU, probably due to a chronic inflammatory status derived from HGA-benzoquinone acetic acid (BQA)-melanin-induced oxidative stress.

In an experimental study on alkaptonuric chondrocytes, the tested antioxidants significantly reduced the production of amyloid and inhibited HGA-induced pro-inflammatory cytokines (Spreafico et al. 2013). This might be the basis for new co-therapies for AKU.

The presence of SAA amyloidosis in the stenotic aortic valve of an AKU patient was demonstrated using Congo Red birefringence and immunofluorescence. Light microscopy revealed a co-localisation of ochronotic pigment and SAA amyloid, tissue calcification, lipid oxidation, inflammation and tissue degeneration (Millucci et al. 2014).

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Coincidence of Alkaptonuric Ochronosis with Other Diseases

23

Jozef Rovenský and Oľga Lukáčová

Content

From metabolic disorders, osteoporosis is found in ochronotic arthropathy of the spine and large joints of the extremities. It is assumed that it is the secondary form of osteoporosis caused by immobilisation of severely affected individuals. Babel et al. (1960) presented a family affected by alkaptonuria, phenylketonuria and congenital cataract. Alkaptonuria sporadically occurs in coincidence with psoriasis. In 1955, we had the opportunity to present unique coincidence of alkaptonuric ochronosis and ankylosing spondylitis in a 51-year-old male patient (Urbánek and Siťaj 1955). Our patient came from the family in which 4 out of 5 siblings had ochronotic arthropathy. On the basis of analysis of clinical and X-ray findings in the area of spine, it could be anticipated that ochronotic arthropathy and AS mutually interact. Our patient had typical ochronotic changes, especially the calcifications of intervertebral discs less pronounced than other patients in the same stage of the disease. Premature rigidity of the spine caused by AS probably prevented development of ochronotic changes expected at his age. On the other hand, in spite of classical AS signs (indistinct sacroiliac joints, ossification of paraspinal ligaments, obliteration of intervertebral joints), the patient experienced inadequately mild pain in the whole

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course of the disease. The results from our long-term observation of a large group of patients reveal that relatively mild pain is typical for ochronotic arthropathy.

Ochronotic arthropathy is a disease associated with degenerative changes in the spine manifested with severe pain in the spine and large joints such as the shoulder, hip and knee joints.

Lumbar and thoracic spine is most frequently affected. Medical finding: significant stiffness of the spine and reduced spine mobility may resemble symptoms present in ankylosing spondylitis. However, X-ray findings are different and typical for each disease. Acute exacerbation of ochronotic arthropathy may clinically resemble rheumatoid arthritis.

The scientific literature describes the coexistence of ochronosis and rheumatoid arthritis or ochronosis and ankylosing spondylitis in 2.4/1,000 and 0.5/1,000 cases, respectively (Ball 1989).

Male patient, born in 1937

Karimzadeh et al. (2009) described the case of a 72-year-old patient whose medical history included an attack of arthritis urica 20 years ago caused by a dietary mistake which was relieved after 7–10 days. Now he has been hospitalised due to pain, swelling and redness of DIP and PIP joints of both hands and due to the presence of white material from a tumorous object on the third and forth finger of the hand. Objective examination was accompanied by pain in the hand joints and spine limited mobility of the shoulder and knee joints, hip joints and lumbar spine, redness and swelling and limited mobility of DIP joints. The examination also revealed bluish black pigmentation of the sclera, auricles and hands.

Urine had a normal colour, but after being left in the light for 30 min, the colour changed to black. Laboratory examinations showed hyperuricaemia. Examination of the white matter obtained from the fingers in depolarizing microscope revealed typical microcrystals of monosodium urate monohydrate. Hand X-ray revealed erosions with sclerotic changes. The lumbar spine contained signs of late stage ochronosis: calcifications and ossifications of intervertebral discs, narrowing of intervertebral space with a vacuum phenomenon and osteoporosis. The treatment included 30 mg of prednisolone daily and after improvement of arthritis 100 mg of allopurinol daily.

The patient showed characteristic radiographic signs of chronic arthritis urica, increase in uric acid in the serum and the presence of tophi. There were also pigmentations on the skin, auricle, sclera, as well as radiographic signs of ochronosis. The presence of uric acid arthropathy is not common in the case of alkaptonuria and ochronosis; nevertheless, this patient fulfilled the diagnostic criteria of both diseases.

Male patient, 54 years old

The clinical picture of ochronotic spondylosis and arthropathy sometimes resembles ankylosing spondylitis. Peric et al. (2007) described a case study of a 54-year-old patient. In 1999 he was examined due to pain in the right knee, lumbar spine and left heel. He had experienced lumbago and the sensation of spine

stiffness, more pronounced after exercising, for 20 years. Gradually, the pain became permanent and was accompanied by knee arthritis and knee and Achilles tendon enthesopathy.

The family history did not include ankylosing spondylitis, ochronosis, seronegative spondyloarthropathy or inflammatory bowel disease, uveitis, conjunctivitis, iridocyclitis, psoriasis or urethritis. In 2000 he developed knee and shoulder joint arthritis; Achilles tendon was affected as well. Objective examination described brown-black pigmentations on auricles, nose cartilage and the sclera. There was also dorsal kyphosis, head and neck protrusion, significant limitation of spine mobility in all directions (Schober 0 cm), decreased scope of mobility and pain of the shoulder joints and hip joints, knee synovitis, left ankle arthritis and tendinitis of Achilles tendon.

Erythrocyte sedimentation rate was 10 mm/h, CRP 8 mg/L (normal level up to 6). Antigen HLA B27 positivity was revealed in laboratory examinations. The concentration of homogentisic acid in the urine was severalfold increased. Urine examination revealed its dark colouration.

X-ray documentation contained a description of quadratisation of thoracolumbar vertebrae, marginal syndesmophytes from Th10 to LI, intervertebral space narrowing, accentuation of thoracic kyphosis, lumbar lordosis reduction, numerous calcifications in the area of intervertebral discs and disc vacuum phenomenon.

MRI revealed erosive inflammatory changes in SI joints – second-grade sacroiliitis, narrowing of joint space in hip joints, subchondral sclerosis and enthesopathic changes in greater trochanters.

The patient has ochronosis with typical clinical and radiological findings and positivity of increased amount of homogentisic acid in urine. Moreover, he meets the New York criteria for ankylosing spondylitis.

Female patient, born in 1952

Balaban et al. (2006) observed a 54-year-old woman with a seronegative spondyloarthropathy. Family history did not include any metabolic disease, diabetes mellitus, hypertension, ulcerative colitis, acute frontal uveitis, trauma and genetic or hereditary disease. For 2 years, the patient suffered from pain and reduced mobility of the lumbar spine. With time, night pain appeared.

Objective examination described black pigmentation in the area of the auricles and on the hands, face, sclera and nails. Arthrological examination revealed strong thoracic kyphosis, loss of lumbar lordosis and flexion in hip and knee joints. The clinical finding resembled ankylosing spondylitis. Pain was also present, as well as reduced scope of shoulder joint mobility and reduction of thorax expansion to 2 cm. Laboratory results revealed higher erythrocyte sedimentation rate and CRP. HLA B27, rheumatoid factor, were not present.

X-ray showed intervertebral disc calcifications as in the case of ochronotic arthropathy. Due to persistent pain in SI joints, a MRI examination was also performed, showing erosive changes associated with bilateral sacroiliitis. The condition was evaluated as the coexistence of ochronotic arthropathy and second-grade sacroiliitis.

Female patient, born in 1930

Kihara et al. (1994) described the case of a 64-year-old female patient whose parents were related, and the mother, grandmother and brother suffered from rheumatoid arthritis. In 1992, the patient started to experience increased pain in their shoulders, left knee and LS spine and pain associated with arthritis of the wrists and small joints of the hands.

Objective examination revealed bilateral brown pigmentation of the sclera, bluish black colouration of the auricle, arthritis of wrists, MTP and PIP joints. Laboratory results showed rheumatoid factor positivity (273 IU/ml) in synovial fluid and positive dark colour of urine in the light.

X-ray showed bone bridging and osteosclerosis of the spine, narrowing of intervertebral space from Thl2 to L2, marginal osteophytes and small erosions in the shoulder and knee joints, osteopaenia and narrowing of the wrist joints. Diagnosis of alkaptonuria and ochronosis with increased secretion of homogentisic acid and pigmentation on the sclera and auricles was confirmed in this patient. Generally ochronotic arthropathic changes are more frequent in large joints and the spine, and the small hand joints remain unaffected. The patient with symmetric arthritis of hand joints, X-ray changes and the presence of the rheumatoid factor fulfilled the diagnostic criteria for the development of rheumatoid arthritis. The described case was confirmed as the coexistence of ochronosis and rheumatoid arthritis.

Recently, we also presented the case of a 50-year-old woman with coincidence of ochronosis and RA with considerable clinical activity and rapid progression of destructive changes on hip and knee joints (Rovenský et al. 2000).

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Differential Diagnosis of the Disease

24

Jozef Rovenský and Oľga Lukáčová

Content

Fresh urine of alkaptonuric patient has a normally pale yellow colour but after prolonged contact with air or with alkaline substances (soap, bleach, ammonia, alkaline cleaning agents containing sodium and/or potassium hydroxide, etc.), it becomes dark grey or black. This is a typical sign for alkaptonuria and nearly always allows distinguishing it from other diseases associated with urine colour changes. For example, in another hereditary disease, congenital erythropoietic porphyrinuria (Morbus Günther) that also occurs early after the birth, urine has a typical red colouration. Similarly in haematuria or haemoglobinuria, urine has a pink to red colour. Urine sediment examination is decisive for making the diagnosis. In bilirubinuria, urine has a red-coffee colour (similar to ale) and dark coffee colour in melanuria. However, urine is always coloured in the fresh condition with the exception of alkaptonuria, in which it becomes darker in the open air after several hours or immediately after adding an alkaline substance.

Ochronosis can be endogenous as in alkaptonuria or exogenous caused by the contact with some chemical substances. Several authors have described yellowish pigmentations on the skin and cartilages induced by carbolic acid that was used for

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the treatment of crural ulcers in the past as well as in other conditions Pick (1906a, b), Pope (1906), Goldenberg (1929), Berry and Peat (1931) and Brogren (1952). This exogenous 'carbolochronosis' was not associated with spondylosis and arthropathy. Thomas and Gisburn (1961) presented exogenous ochronosis on the skin and scleras associated with myxoedema after a long-term administration of resorcinol. Anderson (1947) described pigmentations on the eye conjunctiva and cornea in workers producing hydroquinones. Scleras were dark, and corneal spots caused blindness. Sugar and Waddell (1946) found pigmentations similar to ochronosis on the scleras and cartilages during a long-term use of Atebrine. To differentiate ochronotic changes on the spine from other spondylopathies, the crucial finding is calcification of intervertebral discs that is pathognomonic for alkaptonuric ochronosis. Difficulties in differential diagnostic could occur in chondrocalcinotic spondylopathy, but in this disease, there are typical painful episodes of inflammatory character, calcifications in small joints, especially on the wrists, and spondylopathy with milder course without tendency to ankylosis. In rare cases, calcification of the discs in haemochromatosis needs to be excluded. In case of doubts, urine examination for the detection of homogentisic acid is required as well as comprehensive evaluation of ophthalmological, otological and dermatological findings. Alkaptonuric ochronosis has got such characteristic and polytopic symptoms that more serious differential diagnostic problems usually do not occur. It is more necessary to rule out any other disease that would require more urgent intervention before developing the clinical symptoms.

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Therapy of Alkaptonuria

25

Jozef Rovenský and Tibor Urbánek

Content

Several therapeutic approaches have been used in alkaptonuric patients. The protein origin of homogentisic acid was the base for therapeutic attempts with protein reduction in a diet. In the majority of patients, a decrease of homogentisuria was observed; however, regression of joint symptoms occurred only in rare cases. In order to reduce formation of ochronotic pigment and subsequently to reduce the risk of ochronosis with its harmful consequences especially in musculoskeletal system, some but not all authors reported positive effect is attributed to vitamin C. There are contradictory opinions on effectiveness of vitamin C and diet with protein reduction or their combination. While some authors present improvement of clinical syndromes (Morava et al. 2003; Turgay et al. 2009), other authors did not observe any improvement (Phornphutkul et al. 2002; Suwannarat et al. 2005). High doses of vitamin C decrease excretion of benzoquinone acetic acid, but not of homogentisic acid itself.

Therapeutic advances are aimed at direct pharmacological reduction of formation of homogentisic acid. From the theoretical point of view, it could be possible with nitisinone – triketone herbicide that rapidly and reversibly binds 4-hydroxyphenylpyruvate dioxygenase (50 % inhibition at 40 nM concentration) – enzyme that catalyzes

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formation of homogentisic acid from 4-hydroxyphenylpyruvate. Oral administration of nitisinone (0.1 mg) reduces secretion of homogentisic acid into urine by more than 80 % in murine model of alkaptonuria. In 2005, Suwannerat et al. used nitisinone for the treatment of 9 patients with alkaptonuria. Initial nitisinone dose was 0.35 mg that increased to 1.05 mg/day. Tyrosine plasma concentration increased more than 11-fold after 3–4 months of therapy. In some of the patients, diet with protein restriction (40 g/day) was introduced during the last week of therapy. Tyrosine concentration mildly decreased in these patients. Patients treated with nitisinone experienced significantly milder pain in affected joints just after a week of therapy. Weekly ophthalmologic examinations showed toxicity signs in the cornea. Passage of kidney stones, worsening of symptoms associated with aortic stenosis and elevation of transaminases have been observed.

Gene therapy gets from the phase of model experiments to the stage of clinical application in humans, and it can be assumed that it will possibly be used in the treatment of alkaptonuria. The solution would be the substitution of the missing homogentisate dioxygenase; however, this approach can also bring problems. Possible local abundance of maleylacetoacetate acid that forms from homogentisic acid changes to fumarylacetoacetic acid, under normal circumstances, with the effect of another enzyme – isomerase. In the case of local deficiency of isomerase in the liver, maleylacetoacetate acid can accumulate, resulting in serious hepatic disorders like various types of cirrhosis and other diseases. Administration of recombinant homogentisate dioxygenase requires additional studies of distribution of isomerase and other enzymes involved in the metabolic pathway.

Therapeutic and preventive interventions aimed at ochronotic arthropathy are basically identical with the interventions used for the treatment of degenerative diseases of spine and joints. Nonsteroidal anti-inflammatory drugs, physical therapy and balneotherapy with rehabilitation are used as well as prevention of deformities and surgical procedures if needed. Sonophoresis with NSAID (NSAID gel) is beneficial as well as temporary use of cryotherapy and targeted individual rehabilitation during acute inflammatory irritation symptoms.

Screening of all newly diagnosed children with alkaptonuria is very important as well as their continual follow-up, education on diet and lifestyle and correct selection of sports and especially of suitable work.

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History of Nitisinone (NTBC)

26

Sheth Kamlesh

Content

The clinical use of nitisinone, also known as NTBC, has a fascinating history in the context of modern medical practice. This is a remarkable account of how a weed killer has become the mainstay in the treatment of hereditary tyrosinaemia type 1, a lethal inherited metabolic disorder. The story is still unfolding as nitisinone is now being developed for alkaptonuria.

Tyrosinaemia type 1 was recognised as an autosomal recessive disorder affecting tyrosine metabolism during the 1960s and was treated using diets restricted in tyrosine and phenylalanine (Tyrosinemia Type 2012). In 1977, Lindblad et al. suggested that the severe liver and kidney damage in hereditary tyrosinaemia may be due to an accumulation of succinylacetoacetate and succinylacetone (tyrosine metabolites) and that the primary defect in hereditary tyrosinaemia may be decreased activity of fumarylacetoacetase. With the advent of paediatric liver transplants in the late 1980s and early 1990s, transplant was widely offered as a treatment of tyrosinaemia type 1. However, the clinical development of nitisinone in the 1990s has dramatically changed this practice. Following the observation that plants do not grow well under the Australian bottle-brush plant (*Callistemon* spp.), Stauffer Agrochemical worked on leptospermone, a polyketide natural product from the bottle-brush plant. In 1982 they discovered the herbicidal activity of triketones: 2-benzoylcyclohexane-l,3-diones (personal communication 2012: Professor Ted Lock, Liverpool John Moores University and formerly scientist at ICI/Zeneca).

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Imperial Chemical Industries (ICI) Plant Protection division acquired Stauffer Agrochemical in 1987 and started work on the triketone 2-(2-nitro-4- trifluoromethylbenzoyl) cyclohexane-l,3-dione, abbreviated as NTBC. This is a corn tolerant and grass and broad leaf weed killer which caused bleaching and subsequent death of the plants due to reduced synthesis of plastoquinone (Fig. 26.1).

Animal toxicity studies were carried out at ICI and subsequently Zeneca, which was established in 1993 by demerging of various business units of ICI. These studies demonstrated that rats treated with NTBC developed corneal lesions due to hypertyrosinaemia and high levels of tyrosine in the aqueous humour of the eyes, which disappeared on discontinuing of the herbicide. Although the herbicide programme was abandoned (Lock et al. 1998), it was established that the hypertyrosinaemia was due to inhibition of 4-hydroxyphenylpyruvate dioxygenase (HPPD), an enzyme in the tyrosine catabolism pathway HPPD that converts 4-hydroxyphenylpyruvate to homogentisic acid which is implicated in the pathogenesis of ochronosis seen in alkaptonuria. NTBC was shown to be a potent, time-dependent, tight binding yet reversible inhibitor of HPPD in rats.

ICI/Zeneca's central toxicology laboratory collaborated with Professor Lindstedt to demonstrate that NTBC, in addition to being a rat HPPD inhibitor, was a potent inhibitor of human HPPD as well. Once this was demonstrated, Professor Lindstedt requested ICI/Zeneca to supply NTBC to treat children with tyrosinaemia type I. Following the essential ethical approval, from the University of Gothenburg in Sweden and the Medical Protection Agency (Swedish regulators), ICI/Zeneca cleared the necessary legal/patent formalities, and NTBC was made available to Professor Lindstedt for clinical use. The first patient, a 2-month-old child with acute presentation of tyrosinaemia type 1, was treated in February 1991 (starting dose: 0.1 mg/kg/day, increased to 0.4 mg/kg/day) with good results.

Fig. 26.1 *Callistemon* spp. (Australian bottle-brush plant)

Subsequently four more patients with sub-acute presentation of tyrosinaemia were treated (starting dose: 0.2 mg/kg/day increased to 0.6 mg/kg/day). The results, which were published in Lancet, concluded – 'No side effects were encountered. Inhibition of 4-hydroxyphenylpyruvate dioxygenase may prevent the development of liver cirrhosis and abolish or diminish the risk of liver cancer. Normalisation of porphyrin synthesis will eliminate the risk for porphyric crises. This type of treatment may thus offer an alternative to liver transplantation in hereditary tyrosinemia' (Lindstedt et al. 1992). The study was later further expanded, and a worldwide study was started ('The NTBC Study'). This was coordinated by the team from Sahlgrenska University Hospital (SU), Gothenburg, Sweden, to document the effects of nitisinone treatment in tyrosinaemia type 1 patients. This was an uncontrolled, compassionate use, multicenter trial involving 96 local investigators at 87 different hospitals in 25 countries. On request, nitisinone was distributed from SU to hospitals all over the world on a compassionate use basis. The physicians/investigators sent blood and urine samples for analysis to SU at regular intervals, according to a protocol designed by the research team at SU, together with results of local laboratory tests and clinical information. The NTBC Study period was from February 1991 to August 1997. In 1994 Zeneca sublicensed NTBC to Swedish Orphan AB to commercialise NTBC. From late 1994, the distribution of nitisinone was gradually shifted from SU to Swedish Orphan AB, Stockholm, Sweden. The study patients continued the treatment after the study on a compassionate use basis. The clinical documentation submitted to the European Medicines Agency (EMA) in 2004 consisted mainly of the analysis of the results of the NTBC Study. The safety analysis also included those patients given nitisinone on a compassionate use basis, but not participating in the NTBC Study. Evidence of clinical efficacy was mainly based on the compassionate use in 207 patients enrolled in the NTBC Study. Nitisinone was administered orally twice daily, initially at a daily dose of 0.6 mg/kg body-weight until it was clear that this dose was generally too low. From 1994, 1 mg/kg body-weight was recommended as the total daily initiation dose. The individual response to treatment was evaluated, and the dose was adjusted if considered necessary. No daily dose exceeded 3.0 mg/kg. Nitisinone treatment was always combined with a diet restricted in tyrosine and phenylalanine.

Besides the main analysis regarding the 207 patients who were included between 23 February 1991 and 21 August 1997, a complementary analysis referred to the 250 patients who were included between 1 July 1993 and 28 March 2000, after all investigators had received the recommendations of an initial daily dose of l mg/kg body-weight. Hereditary tyrosinaemia type 1-specific biochemical variables (urine and plasma succinylacetone, erythrocyte PBG synthase and urine 5-ALA), a-fetoprotein, death, liver transplants, hepatocellular carcinoma and porphyria-like crises were evaluated.

On January 18, 2002 the FDA approved (priority approval) nitisinone for the treatment of hereditary tyrosinaemia type 1. Since then, it has been marketed in the USA by Rare Disease Therapeutics (RDT) as Orfadin. On February 21, 2005, under orphan drug regulations, the EMA granted marketing authorisation under exceptional circumstances, for the treatment of hereditary tyrosinaemia type 1 (Orfadin

EPAR 2009). In June 2009, EMA Committee on Human Medicinal Products (CHMP) opined that there were no remaining grounds for the marketing authorisation (MA) to remain under exceptional circumstances, and subsequently the MA was granted with unlimited validity (Fig. 26.2).

Nitisinone is marketed by Sobi (Swedish orphan international, which after its merger with Biovitrum in 2010 became Sobi) in Europe and the rest of the world (excluding the USA – where is it is marketed by RDT) under the trade name 'Orfadin'. Clinical experience includes more than 700 patients, with some patients being on nitisinone for about 20 years and more than 5,000 years of patient-years. The experience has been published in several articles (Lindstedt et al. 1992; Holme and Lindstedt 1995a, b, 1998, 2000).

Furthermore, in addition to hereditary tyrosinaemia type 1, there are other diseases where the potential role of nitisinone has been investigated and may be useful, for example – alkaptonuria (Anikster et al. 1998; Suzuki et al. 1999), neuroblastoma (Kobrinsky and Slojander 2006) and oculocutaneous albinism (Manga and Orlow 2011; Onojafe et al. 2011). Currently, clinical development of nitisinone for alkaptonuria is underway (www.developakure.eu/2013).

In conclusion, since 1992, nitisinone – a compound developed from work on triketone herbicides – has become an effective pharmacological treatment by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (Santra and Baumann

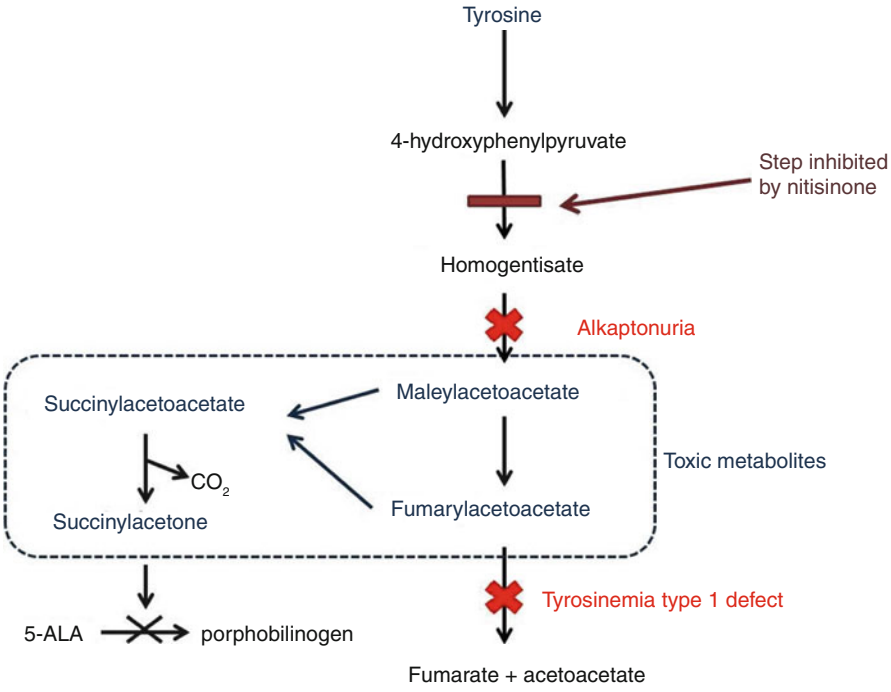

Fig. 26.2 Effect of nitisinone on the metabolic breakdown pathway of tyrosine

2008). Its use in hereditary tyrosinaemia is now well established – and there is potential for treating other conditions. A systematic clinical development programme in alkaptonuria is underway.

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Case Reports

27

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Content

The aim of this chapter is to overview the case reports of patients with alkaptonuria and ochronosis which were collected over the past 60 years. All patients presented in the case reports underwent detailed clinical and joint examination. The following auxiliary examinations were performed in all patients, while most of them were repeatedly checked:

(a) Chest X-ray, ECG
(b) Complete chemical and morphologic urinalysis, particularly focused on detection of homogentisic acid that was also measured quantitatively
(c) Blood count and morphology
(d) Erythrocyte sedimentation rate according to Westergreen (hereinafter referred to as ESR), coagulant range according to Weltmann (hereinafter referred to as Weltmann), reaction according to Takata-Ara (hereinafter referred to as Takata), gross titration test with Hayem's solution (hereinafter referred to as gross), cadmium-sulphate reaction according to Wunderly-Wuhrmann (cadmium reaction) (serum protein electrophoresis in half of the cases) and BWR

†deceased

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(e) Serum BUN, uric acid, calcium, potassium, sodium and chlorides
(f) Glycaemic curve, creatinine clearance
(g) Thorns test with ephedrine
(h) Complete skiagraphic examination of spine and joints

To be brief, we only mention pathological findings, if not stated otherwise, the other results are in reference range. Skiagraphic findings are given separately.

Male patient born in 1915, construction worker; followed at our institute since 1953

Medical History. He noticed urine darkening from childhood; in 1952, his attention was drawn to blue colouration of his auricles. At the age of 32, he felt twitches in the upper part of his thoracic spine while carrying a heavy load with the subsequent pain lasting for half a year, with relief after a stay in a spa. From the age of 37, he noticed slowly worsening pain in the sacral area, especially when straightening from anteflexion, feeling of inelasticity during spine movements, paraesthesia and worsening of the condition after a period of hard manual work. Since the end of 1952, he had mild pain in knees after a physical strain.

Spine. Physiologic curves are indistinct, spine is straightened, sulcus dorsalis persistens in Th8–12 area, Thomayer's sign is 10 cm (hereinafter referred to as Thomayer), Schober's distance is 6 cm (hereinafter referred to as Schober), Stibor's distance is 12 cm (hereinafter referred to as Stibor), Forestier's sign is 0 (hereinafter referred to as Forestier), dorsal flexion is not possible, and respiratory excursions is 6 cm.

Laboratory Findings: Fehling's test ++ (hereinafter referred to as Fehling), Nylander's test + (hereinafter referred to as Nylander), sugar negative by Polarimetrie method. Mean excretion of homogentisic acid in urine: 3 g/day.

X-Ray Skiagraphic Findings

Lumbar Section of the Spine: Narrowing of L5–S1, L1–2 disc spaces. Disc chondropathy with ochronosis signs in L1–2 area. Mild spondylosis of vertebral bodies and smaller lumbar lordosis; disc spaces in thoracic section are discretely narrowed sporadically. Deforming spondylosis is accentuated especially on anterior upper and lower edges of vertebral bodies. Thoracic kyphosis is smaller.

Knee Joints: Articular slit of the left knee is discretely narrowed in medial as well as lateral part.

Male patient, born in 1896, assistant, currently a retired person; followed at our institute since 1945

Medical History. He had scarlet fever in childhood. At the age of 19, he suffered from malaria. At the age of 26, he had pneumonia. In 1950 he had an attack of rheumatic fever; he was treated with salicylate and later with pyridine in full doses. At the age of 39, he started to feel pain in sacral area; firstly, it was periodic, later permanent with irradiation to lower extremities. At the age of 41, he experienced severe lumboischialgia; from that time, he felt numbness in the spine with progressive

limitation of spine mobility. He repeatedly underwent balneologic therapy with good results. At the age of 46, after a long period of working in the kneeling position, he experienced painful oedema of the right knee that lasted for several weeks, pain persisted. At the age of 50, painful limitation of shoulder mobility occurred. At the age of 51, he ceased work, and from that time, the progression was halted, and pain regressed. Rigidity in the area of spine and shoulder remained; knees hurt only after a longer walk.

Physical Examination in 1945: Markedly short figure, 147 cm; skeleton less developed; adequate nutritional status; weight, 47 kg. Posture in mild anteflexion. Auricle cartilages had blue-grey colouration.

Spine. Straightened contours without physiologic kyphosis and lordosis. Short neck with lower hairline. Apparent numbness of the whole spine in anteflexion; bending backwards and lateroflexion are considerably limited; unfolding is minimal. Sensitivity on percussion and deformation of processes were not found. Axial blow is not painful.

Upper Extremities. Slight atrophy of left shoulder muscles; elevation of 70° on the right side, 60° on the left side, abduction of 50° on the right side, 40° on the left side; retroversion is painful.

Lower Extremities. Muscles of pelvic girdle are spastic; dorsal flexion in hips is reduced; abduction of 20°; adduction is free. Knee joints: painful oedema of the capsule on the right side, slight synovial reaction with effusion, mildly reduced flexion, pain in extreme positions.

Laboratory Findings: Fehling +, sugar negative by Polarimetrie method. Head in slight forward projection, hypertonic nuchal muscles, chin-sternum mobility ranging from 2 to 12 cm, Forestier 0, 30° rotation bilaterally, thoracic and lumbar spine straightened to one arch, only minimal lumbar lordosis. Lateroflexion and bending backwards are not possible; Stibor is 3 cm; Schober is 0; Thomayer is 38 cm.

Upper Extremities. Concentric reduction of the right shoulder by about 20 %. Left shoulder: considerable atrophy of the girdle and shoulder muscles, anteflexion 60°, retroflexion 30°, abduction and elevation 50°; rotational movements reduced by 75 %.

Lower Extremities. Flexion in hips, 90°; abduction and rotational movements are only slightly reduced, free adduction, painful forcing of movements, valgus knee deformities, contours of both knees are thickened, atrophy and flabbiness of quadriceps femoris muscles, tenderness of popliteal tendon insertions, right-sided mobility 170-70°, left-sided mobility 160-70°, dry crepitations. Nails are brown in distal parts.

Laboratory Findings. Fehling ++, Nylander +, sugar negative by Polarimetrie method. Mean secretion of homogentisic acid is 2.5 g/day.

X-Ray Skiagraphic Findings. Typical chondropathy of intervertebral discs with apparent ochronosis and subsequent calcification and deforming arthrosis. Diffuse changes along the whole spine, but the most pronounced finding is in lumbar section. Skull X-ray: diffuse decalcification of sella turcica.

Male patient, born in 1922, smelter; followed at our institute since 1952

Medical History: Although he was in generally good health, he noticed urine darkening from childhood. In December 1951, at the age of 29, he experienced sudden severe pain in the muscles of the left calf. In spite of limping while walking, he did not stop working. Later on, he felt a twitching pain in the sacral area, especially during movement initiation. The pain escalated during the last 2 years. He also felt severe pain at rest during the nights and noticed loss of elasticity and rigidity of the spine.

Physical Examination. Shorter figure, 162 cm; weight, 64 kg; athletic type of figure. Hypertrophic tonsils with purulent foci. Significant bradycardia, 48/min. ECG, supraventricular arrhythmia. Horizontal yellow bands of about 2 mm width on both scleras in the slit; phlyctenulosis of the left eye. Auricle cartilages on helix crura have blue-grey shade. Green colouration under the armpits.

Spine. It is markedly flat, straightened, some of spinous processes at Th9–L3 level bulge out; mobility of cervical and upper thoracic spine is free; rigidity escalated in distal direction; lumbar section nearly does not unfold. Stibor 5 cm, Schober 2 cm, lateroflexion 10 cm, Thomayer 30 cm, respiratory excursions 10 cm, retroflexion is reduced, Forestier 0.

Laboratory Findings. Fehling ++, Nylander +, sugar negative by Polarimetrie method. Mean excretion of homogentisic acid is 3 g/day.

X-Ray Skiagraphic Findings

Spine. There is a discrete narrowing of intervertebral discs in distal lumbar section. Ventral half of L5–S1 disc shows initial signs of impregnation by fine granular ochronotic masses. Mild hyperplasia on proximal ventral edge of L4 body.

Knee Joints. Fibular condyles of both femurs show incipient signs of some thickening and squareness with increased sclerotisation of trabecules. These signs have to be considered as incipient signs of articular ochronosis.

Male patient, born in 1891, manual worker; followed at our institute since 1947

Medical history. Due to mental retardation of the patient, data are incomplete and unreliable. He experienced several injuries and was treated due to peptic ulcer. He started to have rheumatic problems at the age of 42; firstly, he only had pain and mobility reduction in the sacral area and spine, later on the hip and knee joints. He also experienced transient knee oedema. Pain was always not severe, dependent on strain. At the age of 52, he became disabled and could walk only with crutches.

Physical Examination. Short figure, 154 cm; weight, 56 kg; skeleton well developed, flabbiness of muscles. Severe disability on the basis of deformation of the spine; the hip and knee joints are apparent at the first sight. Mental retardation, head fixed in 20° lateroflexion. Thyroid gland is enlarged, with nodes. Apparent grey-brown colouration of face, auricle cartilages with blue shade, of more solid consistence. Deep ochronotic spots of 3×4 mm size on both scleras, medially from the cornea. Similar, but less deep, badly demarcated small punctiform spots are present at lateral margins of cornea.

Spine. Bended forward, hyperlordosis of cervical spine with head forward projection, increased thoracic kyphosis with dextroconvex scoliosis, thoracic and lumbar part form one arch with relatively small diameter of curvature, some of lumbar processes bulge out, paravertebral muscles are spastic and atrophic, minimal head movements, 25° rotation bilaterally, Forestier 19 cm, lumbar section does not unfold at all, Stibor 2 cm, Schober 0, lateroflexion 2 cm, Thomayer 52 cm, respiratory excursions 5 cm.

Upper Extremities. Concentric reduction of mobility in shoulder joints by about 30 %, dry marked crepitations, clumsy small movements. Nails have blue-violet shade.

Lower Extremities. Significant retraction in hip joints with the limitation especially of abduction bilaterally, fixed compensatory flexion contracture, more pronounced on the right side. Knee joints deformed, in valgus position, with considerably bulged medial condyles. Flexion contracture at 165° angle, painful flexion up to 90°, very noisy crepitations during movements. Incipient retraction of ankles. Difficult gait with two crutches.

Laboratory Findings. Fehling ++, Nylander +, sugar negative by Polarimetrie method. Mean excretion of homogentisic acid is 3 g/day.

X-Ray Skiagraphic Findings

Shoulder Joints. Chondropathy of both humerus heads in the form of patchy translucency, especially subchondrally with subchondral sclerosis and subsequent flattening of epiphyses.

Spine. Marked ochronotic changes with granular calcification on discs of distal lumbar vertebral bodies as well as on proximal discs. Some of the discs are more massively calcified; others show gradual fragmentation and even pulverisation. Vertebral bodies are flattened, with marginal osteophytes. The spine has a 'bamboo rod' appearance, while the secondary changes of spine in the sense of pathologic deviation cannot be seen.

Pelvis and Hip Joints. There is a particularly pathognomonic finding in the right hip joint, where the femur head is rugged and pycnotic. There are small focal translucencies on the remains of articulation cartilage that is markedly sclerotic. Comparison of X-rays showed that these changes developed during a 2-year period. Two years earlier, there was a patchy translucency of triangle shape in the central part of the femur head. The size was $3 \times 2.5 \times 2.5$ cm with the base facing to articulation cartilage. Simultaneously, 2 years ago, there was a granular translucency at iliac edges of both acetabula as can be seen in immature enchondroma.

Knee Joints. Narrowing of articular slits with marginal appositions, especially on fibular condyles of tibiae and femurs. Demarcated osteosis in the form of patchy enostosis of metaphysis of the left femur.

Female patient, born in 1888, farmer; followed at our institute since 1952

Medical History. Approximately from the age of 30 (unreliable information), she noticed urine darkening in the open air and brown-black spots on underwear. At the age of 38, she started to feel gradually worsening back pain, which regressed after the balneotherapy. At the age of 44, she underwent cholecystectomy. At the age of

50, she started to feel pain, mostly of static character, in the crura, hips and knees. Sometimes she had to stay in bed, walking considerably worsened due to progressive limitation of mobility in her hips and knees. Walking was possible only with crutches. She did not have pain at rest.

Physical Examination. Shorter figure, 150 cm; weight, 68 kg; flabby muscles. Grey-brown face colouration; several blue-black punctiform spots of pin head size on the left cheek. Typical ochronotic changes on sclerotics and auricles. Nails of the left hand have a blue-grey colouration, especially in matrix area.

Spine. Contour is considerably changed, incipient hyperlordosis in cervical section with head forward projection, increased thoracic kyphosis with the top moved in proximal direction, lower thoracic section is flattened with fluent transition to straightened lumbar section. At Th5–Ll level, spinous processes bulge out. 25° head rotation bilaterally, head lateroflexion is not possible, and retroflexion is reduced. Forestier 16 cm. Thoracic and lumbar part of the spine is rigid, with minimal unfolding. Schober 1 cm, Stibor 3 cm, lateroflexion 3 cm, Thomayer 32 cm, respiratory excursions 4 cm.

Upper extremities. Shoulder retraction with concentric limitation of mobility by 50 %; forced movements are painful; Heberden's nodes.

Lower Extremities. Considerable limitation of mobility of hip joints, 90° flexion, 155° extension, abduction and rotational movements are not possible. Knee joints are thickened; parapatellar sulci are smoothed; patellae are fixed; 70–140° mobility bilaterally; dry crepitations; mild ankle retraction; muscles of lower extremities are very flabby.

Laboratory Findings. Fehling ++, Nylander +, sugar negative by Polarimetrie method. Mean excretion of homogentisic acid is 2 g/day.

X-Ray Skiagraphic Findings

Scapulohumeral Joints. Dysostosis and chondropathy of both humerus heads. Thickening of articular cartilage of the right shoulder, narrowing of articular slit more on the right side, demarcated hyperostosis in supraglenoidal area. Flattening of both humerus capitula.

Spine. Very typical and advanced changes of ochronotic calcification of nearly all discs in lumbosacral as well as in the whole thoracic section of the spine. Disc calcification is marked and massive especially in the lumbar region. Some discs in the anterior or posterior half are completely indistinct, and only the central stalk of the disc persists. Adjacent vertebral bodies are completely synostotically connected like by a pseudo-inflammatory fusion. Fusion character is completely different than what we observe in specific or nonspecific spondylitis. There are also very typical ochronotic spondylarthropatic changes in cervical section. It is, namely, narrowing and calcification of the discs, ossifications of intervertebral ligaments and severe deformation of vertebral bodies. Some vertebral bodies are connected by synostosis.

Pelvis and Hip Joints. Both femur heads are markedly flattened, with chondropathic changes. There are numerous patchy translucencies with coarse granular sclerosis and dysostosis in epiphyseal structure. Articular slit is extremely narrowed bilaterally. Hyperostotic reactive changes can be seen on ischiopubic insertion of

the capsule as well as on iliofemoral insertion in the form of irregular calcification bands that partly have osteoid and partly sclerotic structure. There are irregular pseudocystic translucencies on the greater trochanter as well as on the whole trochanteric mass. Similar changes can be seen in the central part of the neck where is a marked translucency of irregular triangular shape of 2 × 2.5 × 2.5 cm size. There is sclerosis in the peripheral part of this translucency and osteoid structure with granular calcification in the centre. A similar finding, but to a lesser extent, is present on the left hip joint. There are considerable irregular bizarre hyperostoses with periosteal incrustations of both ilium bones. Symphysis is ossificated. There are considerable hyperostoses on both alae of the ilium bones, especially at their margins.

Knee Joints. Thickening and angularity of lateral condyles of femur and tibia. Subchondral necrosis with cartilage impregnation by ochronotic pigment. Hyperplastic reactions in the form of button-like osteophytes. Pseudocystic translucency of lateral condyles of tibia and femur and also in the area of medial condyles suggests ostial deposition of ochronotic pigment. Articular slits in lateral part are extremely narrowed, especially on the right side. Deforming arthrotic changes on both patellae.

Male patient, born in 1912, chimney cleaner; followed at our institute since 1952

Medical History. He had Spanish flu in childhood. He experienced frequent nasal bleeding. At the age of 27, he had jaundice and stayed in hospital for 2 months. He had darker urine when he had jaundice; after the recovery, it was lighter, but not as much as previously. From that time, he noticed that urine became darker in the open air. Urine had left dark spots on underwear already before jaundice. At the age of 31, he noticed bluish colouration of auricles that slowly escalated. From the age of 30, he had migrating pain in the shoulders, nuchal ape, hands and sacrum without oedema and fever. Pain, especially in sacrum, became more permanent, dependent on physical strain with the associated clumsiness and inelasticity of spine movements. Recently he had pain of the back and right shoulder.

Physical Examination. Shorter figure, 158 cm; of muscular type; weight, 52 kg; straight posture with incipient trunk anteflexion, pectus carinatum. Grey-brown colouration of face. Small pigmentations in conjunctiva and sclerotic of both eyes in eye slit medially as well as laterally from cornea. Auricle cartilages have grey-sky-blue colour; auricles are harder, less elastic.

Spine. Physiologic curves in thoracic and lumbar section are smoothed, spine is flattened, small-arch kyphotic curve at Th-L transition. Th5–9 spinous processes bulge out. Deepened and persisting sulcus dorsalis at Th11–L3 level. Tenderness on percussion in lower cervical and upper thoracic section; incipient pectus carinatum; respiratory excursions 5.5 cm. Thoracic and lumbar spine is completely rigid. It does not unfold during movements, Stibor 1 cm, Schober 0, Thomayer 30 cm, Forestier 0, lateroflexion 4 cm bilaterally, bending backwards is not possible.

Upper Extremities. Incipient muscular atrophy of pectoral girdles, area of shoulder joints is mildly diffusely tender on palpation; Valleix points are tender on the right side, mild concentric limitation of mobility in the area of the right shoulder

with pain when completing the movement. Elbow flexion is mildly reduced, cracking when completing the movement.

Lower Extremities. Left hip joint: 90° flexion, 45° abduction. The right hip joint, 110° flexion; forced movements are bilaterally painful; slight thickening of the right knee capsule; coarse cracking.

Laboratory Findings. Fehling ++, Nylander +, sugar negative by Polarimetrie method. Mean excretion of homogentisic acid is 3.5 g/day.

X-Ray Skiagraphic Findings

Shoulder Joints. Arch-like pseudocystic translucencies can be seen on both scapulae in supraglenoidal area, more pronounced on the left side. Pseudocystic translucency has the features of enchondroma or dissecting osteochondritis of corn grain size. Smaller usurations with mild sclerosis in adjacent area are displayed on axillar X-ray. Lower part of articular slit is asymmetrical and slightly narrowed.

Spine. Vertebral bodies are markedly porotic. Porosis is most accentuated from the profile projection. Chondropathy and fragmentation of intervertebral discs is typical. These discs are nearly completely crushed, impregnated with ochronotic pigment at some places. Majority of intervertebral spaces is extremely narrowed. The edges of vertebral bodies show discrete spondylosis, especially in thoracic section. Thoracic kyphosis is smaller as well as lumbar lordosis.

Pelvis and Hip Joints. Focal irregular translucencies can be seen in supraacetabular area bilaterally. The translucency has a rhomboid shape of 2 × 2 cm size on the left side, with apparent enostosis in the centre and on the margins. There are three ossification shadows in periosteal area that look like periarticular calcifications of the capsule. There is another more massive shadow of 2.5 × 1 cm size over the iliac part of acetabulum in the proximity of gluteus minimus muscle origin. Another calcification granule is just under the iliac edge of the acetabulum and looks like granule of ochronotic pigment or as a loose fragment after dissecting chondritis in the past. Other osteotic changes are present on both ilium bones and the epiphysis.

Male patient, born in 1951, on disability retirement; followed at our institute since 2007

Spine involvement with massive deposits of ochronotic pigment into intervertebral discs with their successive destruction and development of secondary osteoarthrotic changes is typical in AKU. The most frequently affected extraspinal locations are the eyes, auricles, tendon and ligament insertions in the area of pelvis, femur trochanters, knees and calcanei (Fig. 27.1). Deposits of ochronotic pigment in tendons cause secondary calcification of insertions with high risk of their rupture.

In this case report, we present the occurrence of peripheral enthesopathies in a patient with familial occurrence of ochronosis. We also describe the possibility of using musculoskeletal ultrasonography in the diagnostics of enthesopathies.

Case Report: A 56-year-old patient with the occurrence of familial ochronosis in two elder sisters coming from Záhorie region. The disease initially had painless course; the first clinical symptoms occurred after the age of 40 in the form of Achilles tendon pain bilaterally which are rarely presented early symptom of the disease in literature. Back pain and recurrent oedema of the knees and ankles

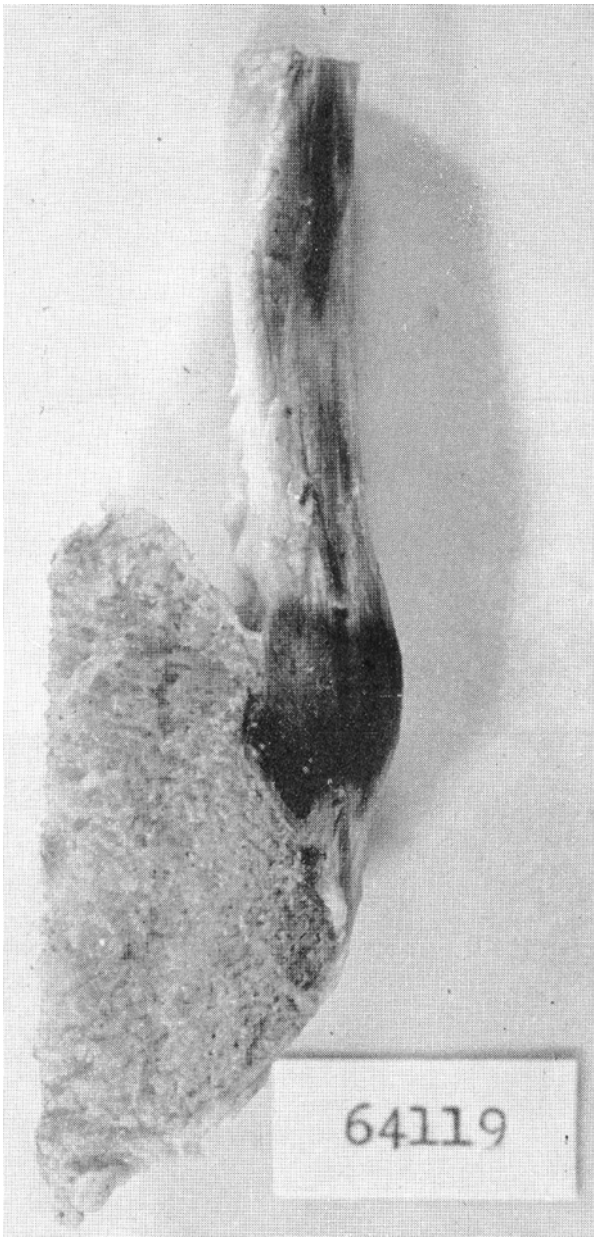

Fig. 27.1 Ochronotic involvement of the tendon at the place of its insertion to the bone

successively appeared. In 2001, the patient underwent arthroscopic synovectomy due to recurrent exudative synovitis of the left knee at the regional orthopaedic department, with the finding of hyperpigmented foci on cartilages. On the basis of arthroscopic finding, typical ochronotic colouration of the scleras, alkaptonuria and positive family history, the patient was diagnosed with ochronosis. From that time,

the patient has been followed by his local rheumatologist as well as at the National Institute of Rheumatic Diseases in Piešťany. In the further course of the disease, back pain was escalating with relatively rapid development of ankylosis on the basis of secondary degenerative changes. The patient also had the signs of bilateral gonarthrosis grade III and coxarthrosis grade II with recurrent plastic synovitis and repeatedly inflamed lymphoedema of the right leg that regressed after the administration of haemorheologic agents and enzyme therapy. In March 2010, he underwent implantation of total endoprosthesis of the right knee due to severe deformity and significant functional limitation.

In June 2010, the patient was hospitalised at the National Institute of Rheumatology Diseases due to recurrent oedema and pain of the left knee. Apart from the above-mentioned painful oedema of the left knee, clinical findings included accentuated kyphotic deformity of the spine with limited mobility as well as worsened mobility in the arms, hip joints and knees. In the left knee, there was a severe pressure pain at the site of insertion of the patellar ligament to the tibia that did not correlate with the extent of clinical finding on the knee. Physical examination revealed typical ochronotic colouration of the right sclera and urine darkening on standing in daylight (Fig. 27.2).

Laboratory examinations showed moderately elevated markers of inflammation (ESR: 35/75, CRP: 12.2 mg/L); blood count, hepatic enzymes, uric acid and renal parameters were in reference range. Negativity of rheumatoid factors, antinuclear (ANA) as well as of anti-citrulline (anti-CCP) antibodies persisted in immunological profile. Radiological examination showed signs of considerable secondary destruction of axial skeleton due to ochronosis with the development of secondary osteoarthrotic changes (Fig. 27.3). There were also bigger enthesophytes in the area of greater trochanters (Fig. 27.4) and one small enthesophyte on the upper pole of patella of the left knee (Fig. 27.5). In the context of differential diagnostics of the left knee involvement with dominant clinical symptoms, we performed

Fig. 27.2 Alkaptonuric colouration of urine after a longer stay at daylight

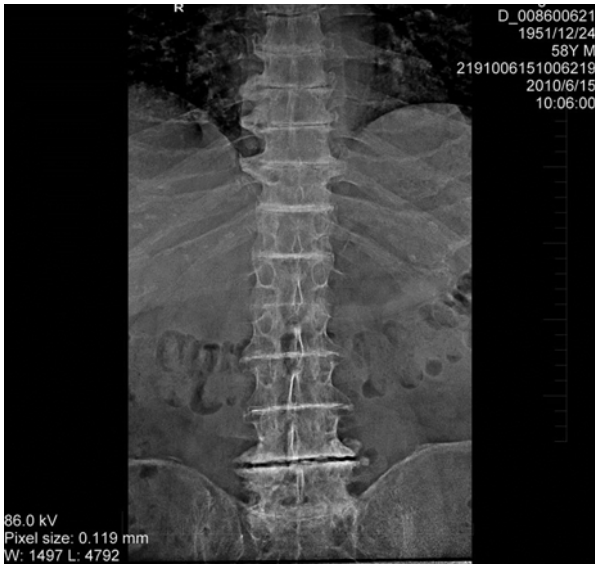

Fig. 27.3 X-ray of thoracic spine with narrowed hyperpigmented intervertebral discs and secondary osteoarthrotic changes

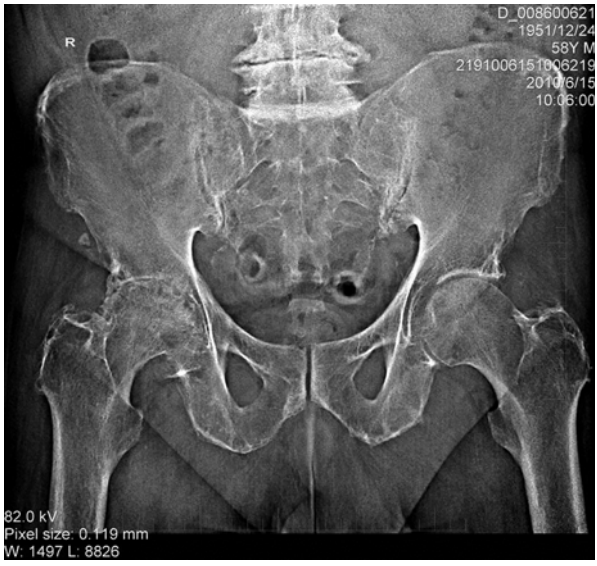

Fig. 27.4 Pelvis X-ray with narrowed articular slits of hip joints, bigger enthesophytes on femur trochanters and fibro-ostoses on the alae of ilium bones of even hyperostotic character

musculoskeletal ultrasonography which revealed small anechogenic effusion in the area of suprapatellar recesses with parietally thickened synovium up to 3 mm, more echogenic and reduced intra-articular cartilage and marked enthesitis affecting patellar ligament. At the place of insertion of patellar ligament to tibial tuberosity, ultrasonography showed considerably widening of the tendon up to 7.3 mm, which was hypoechogenic with typical loss of fibrillar structure and anisotropy, with small hyperechogenities and enthesophytes along it and higher congestion during power

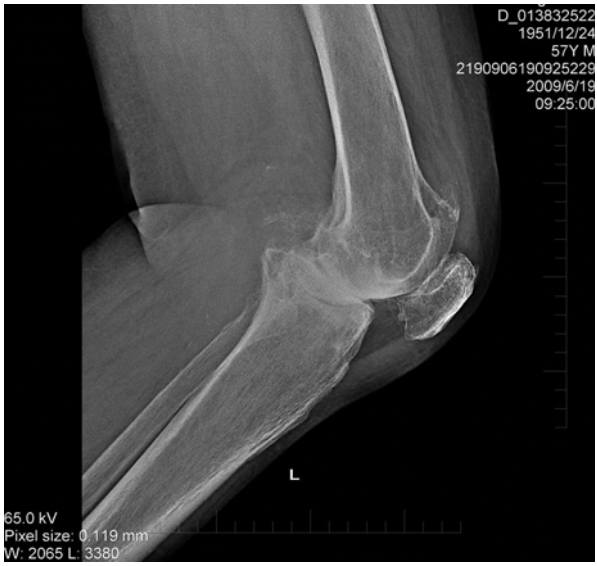

Fig. 27.5 X-ray of the left knee with narrowed articular slit, marginal osteosclerotic changes and small enthesophyte on the upper pole of patella

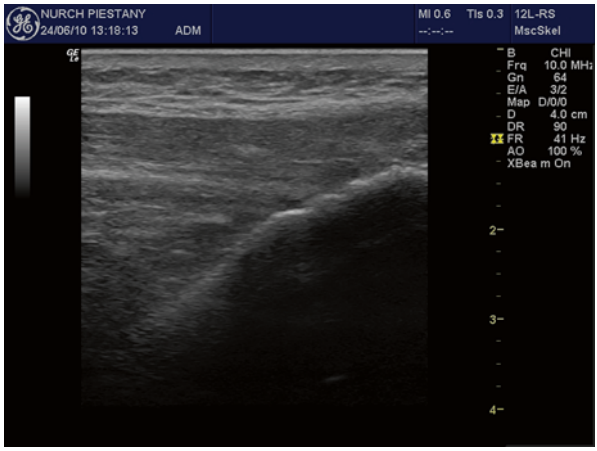

Fig. 27.6 Ultrasonography of the left knee. Widening of the tendon of patellar ligament that is hypoechogenic with typical loss of fibrillar structure and anisotropy. Small enthesophytes and fine hyperechogenic calcifications at the place of insertion on tibial tuberosity

Doppler mapping (Fig. 27.6). Admixture of hyperechogenities along the tendon was very probably caused by secondary calcifications as the consequence of calcium deposition to ochronotically widened and pathologically changed tendon. The real cause of marked enthesopathy would be clarified by targeted histological examination that was not performed due to ethical reasons. Ultrasonographic examination is a non-invasive, patient-friendly and cheap method for verifying the severe involvement of insertions that often dominates in clinical findings of this relatively frequently diagnosed disease.

NSAID of diclofenac type in combination with vitamin C was continued in medication; local therapy of the left knee consisted of NSAID gel phoresis, cryotherapy and individual rehabilitation. Comprehensive therapy and rehabilitation resulted in mitigation of the symptoms of acute enthesitis.

In this case report, we wanted to highlight the frequent occurrence and problems of enthesopathy in patients with ochronosis – the topic that has been presented only rarely in the literature. In these cases, polymers of homogentisic acid deposit excessively in the structures of locomotor system. In the highly developed forms of the disease, insertions are mostly affected, resulting in considerable clinical problems. The use of more recent imaging methods like musculoskeletal ultrasonography or MRI can clarify the cause of problems in these patients. High-frequency ultrasonography with at least 10 MHz linear probe should be used. The higher the frequency, the better the imaging of more superficial structures. The advantage can be the use of 'power Doppler' mapping to detect increased irritation congestion at the place of insertions. Therapeutic options comprise analgesics or NSAID, NSAID gel sonophoresis. In acute inflammatory irritation signs, cryotherapy and targeted individual rehabilitation can be temporarily used.

Female patient, born in 1954, nun; followed at our institute since 1996

The clinical picture of alkaptonuria (AKU) and ochronosis, its manifestations on the eyes, ears, skin, visceral organs as well as the most serious effects on the locomotor system, ochronotic arthropathy, are well known. The working group of Siťaj, Červeňanský and Urbánek dealt with this disease (Siťaj (1947), Urbánek and Siťaj (1955), Červeňanský et al. (1959), Siťaj (1963), Siťaj and Lagier (1973), Siťaj et al. (1956), Rovenský et al. (2000)) who paid particular attention to genetic aspects of this metabolic disorder. Ochronotic arthropathy is basically a degenerative process of known genesis with marked tendency to disability. Pathological changes on the spine are the most important. Ochronotic pigment is deposited in intervertebral discs; they lose their elasticity with subsequent secondary calcifications resulting in the typical X-ray finding that is pathognomonic for alkaptonuric ochronosis. There are typical degenerative changes on the shoulders, hip joints and knees with the picture of severe and rapidly progressive osteoarthrosis. Peripheral joints of the hands and feet are not affected by ochronotic process. Two cases of coexistence of ochronosis and rheumatoid arthritis (RA) have been presented in the literature yet (Kihara et al. 1994). The scarce information in the literature suggests that the ochronotic process can mask the symptoms of RA and make its diagnosis difficult (Kihara et al. 1994). In our case report, which is the first one in the domestic literature, we would like to point out the coincidence of RA and ochronosis that had a malignant course. Simultaneously, we would like to draw attention to some differences found in the genetic analysis of our patient.

Family history revealed that one sister suffered from alkaptonuria and another one from rheumatoid arthritis. The onset of joint problems dates back to 1974, i.e. after the age of 50 when the patient noticed shoulder pain with successive mobility limitation as well as knee and back pain. In 1981 clinical and radiological examination led to the diagnosis of AKU with the symptoms of ochronotic

arthropathy. In 1982 hip joint pain occurred, more pronounced on the right side, and due to relatively rapid progression. The patient had a right-sided total endoprosthesis implanted in 1983 and the left one in 1985. Since 1985, the patient experienced marked knee pain with condition worsening. During the first hospitalisation at the National Institute of Rheumatology Diseases in 1996, we detected plastic synovitis of the knee with left-sided effusion. Lab parameters: LFT, 5100; HT, 0.5; and ESR, 35/66. The patient had a right knee total endoprosthesis in 1996; 1 year later, the same procedure was performed on the left knee with good post-operative course. In 1996 the patient experienced firstly pain and later oedema of small joints of the hands, and she markedly lost weight. Arthritis signs were not present on X-rays. At admission to our institute in 1997 (after the left knee total endoprosthesis implantation), the patient complained of pain of the small joints of the hands and wrists associated with marked morning stiffness, clinically present arthritis in the above-mentioned joints and with high positivity of anti-globulin reaction (RF) LFT, 2120, and HT, 224. Radiological examination of the hands and feet suggested RA stage III (Figs. 27.7 and 27.8). With regard to the disease activity, the patient started to receive methotrexate 7.5 mg weekly, and she continued to take it until hospitalisation in 1999. On admission, clinical findings were dominated by marked wrist synovitis, MCP joints showed ulnar deviation and the patient had elbow synovitis and morning stiffness lasting for several hours. Acute phase reactants were mildly elevated on admission, but they increased in the course of hospitalisation. Clinical analysis on July 29, 1999, confirmed the malignant form of RA stage IV with marked progression and lytic changes (Fig. 27.9 a, b dated 1999) in spite of continual therapy with methotrexate 7.5 mg weekly. MTX dose was increased to 10 mg weekly in combination with sulphasalazine 2 × 1 tablet and Sandimmun Neoral 3.5 mg/kg/day. The malignant course of the disease is manifested by the clinical course, radiological progression and the need of intense therapy (MTX, cyclosporine, sulphasalazine). From the point of immunogenetic features, the patient had the following HLA antigens: HLA-A2, 11; B-18, 35; Cw4, w7; DR11, 14 52; DQ7.

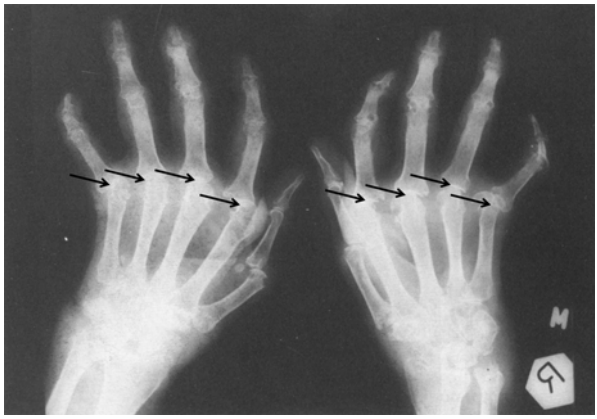

Fig. 27.7 Hands show unequal thickening of soft tissues, ulnar deviation of MCP joints, significant narrowing of interarticular spaces and erosions in MCP joints (*arrows*), small cyst-like defects in proximal interphalangeal joints and marked worsening of X-ray findings on the second and third DIP joint on the left hand and on the fifth DIP joint on the right hand

In this report, we present a patient with diagnosed ochronotic arthropathy in whom lytic form of RA with relatively aggressive progression and insufficient response to MTX therapy developed. Due to the malignant course of the disease, we were forced to administer intense therapy with cyclosporine A, sulphasalazine and increased MTX dose. This therapy and surgeries (4 total endoprostheses) could be

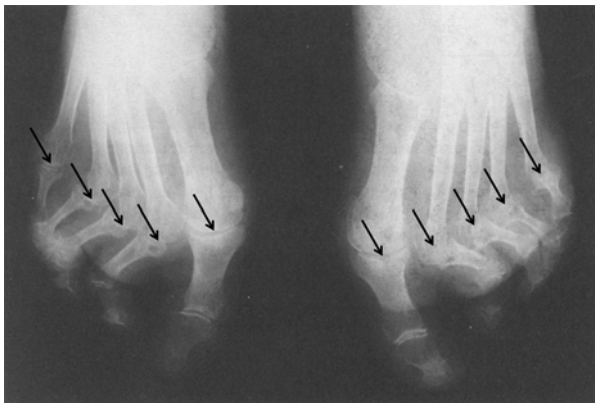

Fig. 27.8 X-rays of feet show progressive fibular deviation and subluxation in MTP joints of both feet (*arrows*) and numerous cyst-like subchondral defects, especially on the second and fourth MTP joints on the right foot and with erosions on the left MTP joints

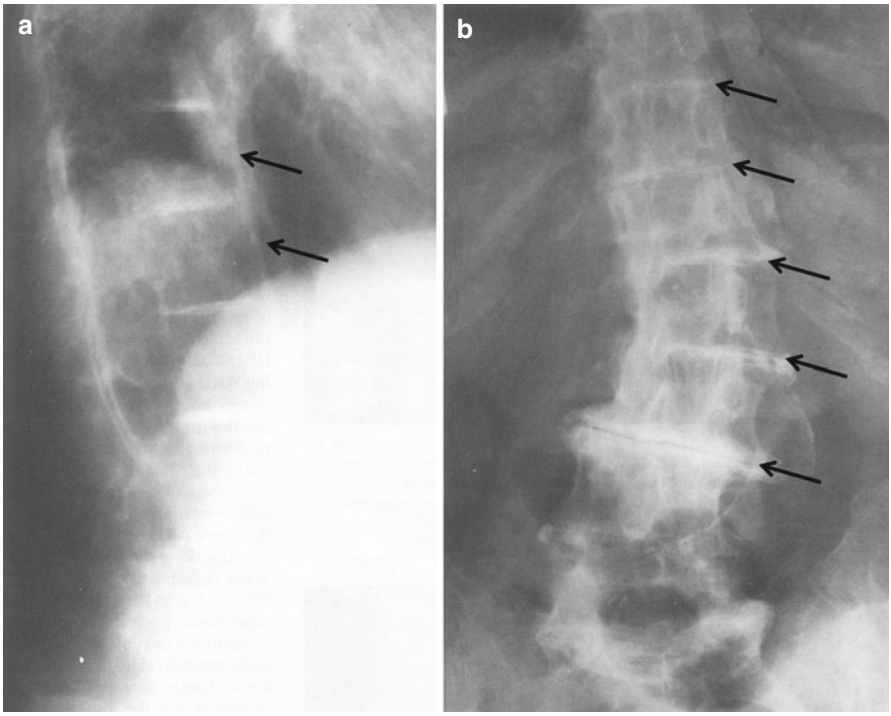

Fig. 27.9 (**a**, **b**) Osteoporosis and scoliosis is present in lumbosacral spine, intervertebral discs are markedly narrowed (*arrows*), calcifications and vacuum phenomenon are found in the remains of intervertebral spaces

the aggravating factors of RA development, because release of some stress hormones like prolactin and growth hormone does not rule out this option. Genetic analysis is also interesting because RA and AKU have genetic components, which are well defined on the molecular level in the case of AKU. In RA attention is mostly paid to immunogenetic analysis with the emphasis on HLA-system antigens. The patient had the above-mentioned HLA antigens. HLA-DR1 and HLA-DR4 antigens that are associated with RA in Slovak population (Bošák 1996) were missing in her genotype. It means that the patient had the form of disease that is not associated with HLA antigens typical for RA. However, it has to be noted that all HLA antigens that represent significant immunogenetic risk for RA development have the common epitope at the amino acids 67–74 (Bošák 1996). Except some subtypes of HLA-DR4 and HLA-DR1, HLA-DR14 belongs to this group of antigens as well. The last mentioned antigen was found in our patient. In spite of the fact that this association is not typical for the Slovak population, it is not possible to rule out immunogenetic effects contributing to RA development. It is interesting that severe forms of RA are usually associated with HLA-DR4 antigen and the patient is HLA-DR4 negative, while having lytic form of RA with rapid progression. From the genetic point of view, it has to be noted that patient's sister had RA. RA can have familial occurrence, but it is not a frequent finding. This fact suggests not only the role of genetic factors in RA development, but also it can have significant impact on severity and course of RA as probably in this case. In case of AKU, the defect has autosomal recessive trait and gradually leads to ochronosis development. The AKU gene is localised on the short arm of chromosome 3, and many mutations causing AKU have been found (Fernandez-Canon et al. 1996). One of patient's sisters suffered from AKU and ochronosis. It is interesting that the family did not come from isolated regions of Slovakia where AKU occurrence is relatively frequent.

From the practical point of view, it seems that it is always necessary to consider the coexistence of two clinically limited forms of rheumatic diseases (Rovenský et al. 2000). Diagnosis of ochronotic arthropathy was clear on the basis of the clinical course. The presence of symmetrical arthritis of the small joints of the hands, synovitis of the right elbow with typical radiological findings and high seropositivity of RF supported the anticipation of RA development. In the clinical course, ochronotic arthropathy preceded RA development. Pain of the shoulders and knees since 1974 with knee synovitis, elevated ESR and positive LFT (5100) and HT (56) detected in 1996 could be the signs of masked RA, the malignant form of which became manifested in 1997. As we mentioned at the beginning of this case report, ochronotic arthropathy presented with severe degenerative changes on spine and large joints, requiring implantation of 4 total endoprostheses (hip and knee joints). In this case report, the coexistence of ochronotic arthropathy and rheumatoid arthritis is described. Ochronotic arthropathy preceded RA development, and its course probably overlapped with RA for several years. RA development occurred in a genetically predisposed individual; it had a malignant course with lytic changes on the small joints of hands with high seropositivity of rheumatoid factors. Ochronotic arthropathy did not mitigate the course of RA.

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